

miR-130b Acts as an Oncogene and Prognostic Biomarker of Breast Cancer: A Bioinformatic Analysis

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Abstract

Background: Breast cancer (BC) is the most prevalent cancer among females globally. microRNAs (miRNAs) could regulate the expression levels of cancer-related genes through binding with target mRNAs. In various cancers, the abnormal expression of miR-130b has been detected. We aims to investigate the molecular mechanism and biological function of miR130b in breast cancer.

Methods: We obtained two microRNA expression profiles from the Gene Expression Omnibus (GEO) database, including GSE45666 and GSE26659. We identified differentially expressed miRNAs (DE-miRNAs) between BC tissue and normal breast tissue based on the GEO2R web tool. DE-miRNAs were filtered by significant prognostic value resulting from Kaplan–Meier plotter. We used the JASPAR database to explore upstream regulators of miR-130b. The potential molecular mechanisms of miR-130b correlation genes were revealed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis in WebGestalt. Protein–protein interaction (PPI) network of miR-130b target genes was constructed by STRING. Cytoscape software was used to visualize the PPI network and hub genes.

Results: miR-130b was highly expressed in breast cancer tissues, which positively correlates with poor prognostic. JASPAR revealed THAP11 might be the upstream regulator of miR-130b. In addition, GO, and KEGG pathway revealed that miR-130b positively regulated PFKF, STAT1, SRC, and NOTCH2, participating in the Thyroid hormone signaling pathway. The PPI network further identified that AR, KIT, and ESR1 as hub genes in BC development.

Conclusion: miR-130b, which is regulated by THAP11, acts as an oncogene and prognostic biomarker in BC by mediating the Thyroid hormone signaling pathway and potential target genes. miR-130b might be a novel therapeutic target for BC treatment.

Introduction

A recent study showed that 276480 American women were diagnosed with breast cancer (BC) in 2020, ranked the most prevalent cancer in female cancers, and caused the death of 42170 women (Siegel et al. 2020). The incidence and death rate of BC increase exponentially with aging (Miller et al. 2020). Despite recent progress in prevention and treatment, still many people died from BC each year (Kan et al. 2018). Therefore, identifying specific molecules that can serve as tumor biomarkers for detection and molecular pathways to provide potential therapeutic targets is critical for BC management.

MicroRNAs (miRNAs) are small non-coding single-stranded RNA molecules that are involved in regulating gene expression via targeting mRNAs (Kotyla and Islam 2020; Stevic et al. 2020). miRNAs participate in crucial biological processes, and their dysregulation leads to the development of various diseases, including breast cancer. miRNAs can function as an oncogene or a tumor suppressor (Ma et al. 2019). MiR-130b, a mature miRNA located at chromosome 21, plays an oncogenic role in multiple cancers, including oesophageal squamous cell carcinoma, liver cancer, gastric cancer, bladder cancer, lung cancer,

and prostate cancer (Chen et al. 2018; Fort et al. 2018; Liu et al. 2018; Wang et al. 2019; Yu et al. 2015; Zhang et al. 2019; Zhu et al. 2019). Conversely, miR-130b plays a tumor suppressor role in ovarian cancer, pancreatic ductal adenocarcinoma, and thyroid carcinomas (Fukuhisa et al. 2019; Paudel et al. 2016; Yip et al. 2011). The relationship between miR-130b and BC remains elusive and required to elucidate further.

The Thyroid hormone signaling pathway could regulate several crucial developmental and physiological processes, including cell growth, metabolic homeostasis, and differentiation, particularly affecting certain tumors (Anyetei-Anum et al. 2018; Bianco et al. 2019; Ma and Ding 2016). A previous study confirmed that hypothyroidism patients had a decreased prevalence of BC; otherwise, patients with hyperthyroidism are likely to suffer from aggressive BC (Liu et al. 2019). Numerous studies have documented the mechanisms of Thyroid hormone (TH) in BC. Marina et al. demonstrated that TH controls BC cells' movement through Integrin α V/ β 3/SRC/FAK/PI3-Kinases (Flamini et al. 2017). Ivonne et al. (Uzair et al. 2019) suggested that TH regulates BC's metastasis by controlling the signaling pathway, which induced integrin α v β 3 to FAK/paxillin/cortactin/N-WASP/Arp2/3 that converge in cell motility. As stated above, it has been proven that the Thyroid hormone signaling pathway is associated with BC cell. However, the relationship between miRNAs and the TH signaling pathway, which promotes BC's evolution and expansion, is still unclear.

This study demonstrated that miR-130b was upregulated in BC tissues by analyzing two microRNA expression profiles in Gene Expression Omnibus (GEO). To get a deep insight into underlying mechanisms contributing to BC, we evaluate the relationship between miR-130b expression and prognostic value. Furthermore, we explored the correlation genes. THAP11, as a transcription factor, was identified to be an upstream regulator of miR-130b. The results of GO and KEGG pathway analysis demonstrated that miR-130b might regulate the TH signaling pathway in BC. In addition, a Protein–protein interaction (PPI) network was constructed to investigate hub genes. These results provide a novel therapeutic target for BC and ameliorating the prognosis.

Methods

Detection and identification of DE-miRNAs

We obtained two microRNA expression profiles (GEO: GSE45666 and GSE26659) from the GEO database (<http://www.ncbi.nlm.nih.gov/geo/>), for the current study. Two profiles were analyzed by an interactive web tool named GEO2R (<http://www.ncbi.nlm.nih.gov/geo/geo2r/>). DE-miRNAs were identified in normal breast tissue and BC tissue by $|\log_2\text{Fold change (FC)}| \geq 1.5$ and adjusted P-value < 0.05 filtering. Common DE- miRNAs were filtered out by Venn analysis (<http://bioinformatics.psb.ugent.be/webtools/Venn/>).

Survival analysis

The Kaplan–Meier plot (<http://kmplot.com/analysis/index.php?p=background>) was used as a web tool, containing survival data of 21 cancers based on GEO, EGA, and TCGA databases. That investigate and

verify the prognostic value of 54k genes (mRNA, miRNA, protein). miRNA BC database was applied to evaluate the prognostic values of DE-miRNAs. Numerous quantile expressions of DE-miRNAs were used to divide the BC patients into two groups. DE-miRNAs with P-value<0.05 were considered to be statistically significant.

Prediction of upstream regulators

We investigated the relationship between miR-130b and genes obtained from the TCGA-BRCA cohort by using the LinkedOmics database (<http://www.linkedomics.org/admin.php>). This database involves multi-omics data within or across 32 types of cancer, including breast cancer. Results were identified by Pearson's correlation analysis. JASPAR (<http://jaspar.genereg.net>) is an online database that stores transcription factor (TF) binding profiles, was used to predict the TFs of CDCA8.

Extracted over-expressed genes of breast cancer from the TCGA database

GEPIA (<http://gepia.cancer-pku.cn>) is a web server that supplies multiple capacities, including tumor, normal differential expressed investigation, and interactive gene analysis. GEPIA was used to compare the median expression of miR-130b in BC and normal samples and to analyze the correlation between CDCA8 and TFs. Furthermore, overexpressed genes in BC were explored by GEPIA.

Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis

The correlated genes with miR-130b from miRWalk (<http://miRwalk.umm.uni-heidelberg.de>), which were input into an open-access web tool, named WebGestalt (<http://www.webgestalt.org>), for GO and KEGG pathway enrichment analysis. The functional enrichment network graph was drawn. GO analysis contains three terms, including biological process (BP), cellular component (CC), and molecular function (MF). We performed a KEGG pathway analysis to identify possible pathways that involved miR-130b correlated genes. False discovery rate (FDR) ≤ 0.05 were regarded as statistically significant.

Exploration of the target genes of miR-130b

Four web servers were selected to explore the target genes of miR-130b, including miRWalk, miRDB (<http://mirdb.org>), Targetscan (http://www.targetscan.org/vert_72/), and DIANA-mT (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=microT_CDS/index). Those common genes which were determined by all four prediction software were selected as potential target genes.

PPI network construction and hub genes identification

To further explore the downstream targets of miR-130b, a PPI network was generated via the Search Tool for the Retrieval of Interacting Genes database (STRING) (<https://string-db.org/>). Genes in the network were reduced by searching overlapping genes between potential target genes of miR-130b and over-expressed genes in BC. Cytoscape software (www.cytoscape.org/) was selected to construct and

visualize the PPI network. We used one plugin in cytoscape (CytoHubba) to compute each overlapping genes, hub genes in our study's hub genes were the top three genes.

Statistical analysis

Prism software (GraphPad, CA, USA) was used to perform statistical analysis. t-test was used to analyze the differences between and among groups. * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$ were considered statistically different

Results

DE-miRNAs Investigation

Two microRNA expression profiles (GSE45666 and GSE26659) were selected to investigate DE-miRNAs between BC tissue and normal breast tissue. As shown in Table 1, the GSE45666 dataset included 101 BC samples and 15 normal breast samples, GSE26659 dataset was composed of 77 BC samples and 17 normal breast samples. After using the GEO2R program to analyze microRNA expression, we obtained 129 and 257 DE-miRNAs from GSE45666 and GSE26659 datasets (Fig. 1). 10 common upregulated DE-miRNAs are shown in the Venn diagram (Fig. 2).

The prognostic value of miR-130b in BC

The prognostic value of ten common DE-miRNAs in BC was obtained from Kaplan–Meier plotter (Fig. 3a–J). After evaluating the overall survival of the DE-miRNAs in BC, two out of ten microRNAs showed statistical significance, including miR-130b ($p=0.012$) and miR-21 ($p= 0.004$). Previously numerous studies are reported on the relationship of miR-21 and BC, however, these studies did not focus on the molecular mechanisms of miR-130b in BC, so we selected miR-130b as our focal point. The results demonstrated that the median survival time of highly-expressed miR-130b patients (124.53 months) was dramatically shorter than low-expressed miR-130b patients (215.20 months). miR-130b was highly expressed in BC tissue than normal breast tissue (Fig. 3k, l).

miR-130b is closely related to CDCA8

Next, we explored the correlation genes with miR-130b (Fig. 4a). Top 50 positively and negatively correlated genes are visualized in Fig. 4b and Fig.4c. As shown in Fig. 4b and Fig. 5a, CDCA8 performed the highest pearson-correlation with miR-130b (Pearson-correlation = 0.6502, $P < 0.01$). CDCA8 expressed significantly higher in BC tissue compared to normal breast tissue based on GEPIA (Fig. 5b), which is in accordance with miR-130b.

THAP11 plays a key role in the upstream regulation of miR-130b

The -0516 ~ -2615 bp region was defined as a potential promoter region of the CDCA8 gene. By analyzing JASPAR data, THAP11 had the highest match score with CDCA8 among all predicted transcription

factors (Fig. 6a, b). To further understand the correlation between THAP11 and CDCA8, we analyzed the data on GEPIA. The results demonstrated that CDCA8 had a positive correlation with THAP11 ($P < 0.01$) (Fig. 6c). Collectively, these data indicated that THAP11, as a TF, is a potential regulator that binds to and initiates the transcription process of CDCA8.

Functional and pathway analysis of miR-130b correlated genes

Based on the GO term analysis, we used Go Slim to provide a general overview (Fig. 7). Gene Set Enrichment Analysis (GSEA) was conducted to further elaborate the GO terms, showing that genes correlated with miR-130b were predominantly enriched in serotonin receptor signaling pathway (GO:0007210) for biological process (BP); receptor complex (GO:0043235) for cellular components (CC); neurotransmitter binding (GO:0042165), serotonin receptor activity (GO:0099589), passive transmembrane transporter activity (GO:0022803), phosphatidylinositol biphosphate kinase activity (GO:0052813), cyclin-dependent protein kinase activity (GO:0097472), structural constituent of ribosome (GO:0003735) and isoprenoid binding (GO:0019840) for molecular function (MF) (Table 2). As shown in Fig. 8a, the KEGG pathway enrichment analysis results demonstrated that the correlated genes were significantly involved in several pathways, including the Thyroid hormone signaling pathway, ribosome, human immunodeficiency virus 1 infection. TH signaling pathway is shown in Fig. 8b. Red plots indicated the miR-130b correlated genes. The significant KEGG pathway enrichment plots are shown in Fig. 9.

miR-130b positively regulated PFKP, STAT1, SRC, and NOTCH2

We selected four well-characterized genes for further studies, which are involved in the TH signaling pathway, including STAT1, SRC, NOTCH2 and PFKP (Fig. 8b). The correlation between miR-130b and four genes was validated by using the LinkedOmics database. The results revealed that miR-130b positively regulated the expression levels of PFKP (Pearson-correlation = 0.3212, $p < 0.01$), STAT1 (Pearson-correlation = 0.1983, $p < 0.01$), SRC (Pearson-correlation = 0.1953, $p < 0.01$), and NOTCH2 (Pearson-correlation = 0.0760, $p = 0.03718$). (Fig. 10).

AR, KIT and ESR1 were hub genes in the PPI network

We selected 323 common genes as the potential target genes of miR-130b (Fig. 11a). Based on the GEPIA database 3554 predicted over-expressed genes in BC were obtained, among which 54 genes were validated in the overlapping regions (Fig. 11b). Protein–protein interaction network is shown in Fig. 11c. We identified hub genes with a connectivity degree in the PPI network (Table 3). The top three genes, AR (degree=9), KIT (degree=7) and ESR1 (degree=7) were defined as hub genes (Fig. 11d), followed by ZEB2 (degree=6), HSPA8 (degree=5), and MET (degree=4). These hub genes were all upregulated in BC.

Discussion

Dysregulation of miRNAs plays key roles in the initiation, differentiation and migration of several types of cancer, including BC (Bertoli et al. 2015). Our study aims to improve BC prognosis by validating DE-miRNAs in BC tissue compared with normal tissue and elucidate the mechanisms regulating particular target genes and pathways. In the current study, miR-130b was identified to be significantly over-expressed in human BC tissue than adjacent normal tissue. After verifying the expression, we investigated the overall survival of miR-130b in BC by using the Kaplan–Meier Plot, which indicated that miR-130b was positively correlated with a worse prognosis. These results elucidated that miR-130b might function as an oncogene when overexpressed in BC. Ana The LinkedOmics database results indicated a strong positive correlation between CDCA8 and miR-130b. We found THAP11 as a transcription factor regulating CDCA8. Accordingly, we hypothesized that THAP11 played key roles in the upstream regulatory mechanism of miR-130b, and regulated the expression level of miR-130b.

THAP11, also known as thanatos associated protein 11, belongs to a unique family of TFs, which recognized particular DNA sequences through atypical zinc finger motif and regulated cell cycle and cell growth (Cukier et al. 2016). THAP11 has been linked and differentially expressed in several cancers, such as gastric cancer, esophageal cancer, and colon cancer (Jin Y 2019; Parker et al. 2012; Zhang et al. 2020). Until now, the function of THAP11 in BC remains elusive.

Next, we attempted to explore the downstream regulatory mechanism of miR-130b in breast cancer via predicting and analyzing genes correlated with miR-130b. A series of functional analysis was used to identify the biological functions and related signaling pathways of miR-130b in BC. Based on the data of GSEA, miR-130b was positively upregulated genes in the TH signaling pathway, such as PFKP, STAT1, SRC, and NOTCH2. Our results suggested that miR-130b was over-expressed in BC, which upregulated oncogenes, including PFKP, STAT1, SRC and NOTCH2, and played an essential role in BC progression.

In our study, PFKP was analyzed as the most positively upregulated gene target in the TH signaling pathway with miR-130b (Pearson- correlation = 0.3212, $p < 0.01$) in BC. PFKP, also known as phosphofructokinase platelet, is the platelet isoform, which encodes a phosphofructokinase A family protein. PFKP might be a significant mediator for cell metabolism, participating in cancer metastasis and initiation (Lang et al. 2019). Recently, PFKP is recognized for high epidemic property in several kinds of aggressive tumors such as glioblastoma and BC (Lee et al. 2017; Moon et al. 2011). In addition, PFKP is closely related to cell survival of muscle-invasive bladder cancer (Sun et al. 2016). Wang J et al. (Wang J 2016) reported that high mRNA expression levels of PFKP were correlated with amassment of apoptotic proteins and cell proliferation in renal carcinoma, indicating the relationship between PFKP and the growth of renal carcinoma. Moreover, lots of evidence suggested that PFKP act as an oncogene in lung cancer and hepatocellular carcinoma (Park et al. 2013; Wang et al. 2015). Our study disclosed a novel mechanistic interaction between miR-130b and PFKP, supposed that the BC progression may be reduced by down-regulating miR-130b, which reduces the expression level of PFKP and constrains the cell initiation and metastasis.

Apart from PFKP, the other three oncogenes associated with miR-130b, including STAT1, SRC and NOTCH2 were worthy of conducting further investigations. Previous studies reported that most of them play a significant role in cell proliferation and differentiation by up-regulating in various human tumors and are closely associated with their overall survival. The expression of STAT1 is related to cell growth, regulation, immune evasion and metastasis (Ryan et al. 2020). Zellmer et al. reported that STAT1 is a latent therapeutic target for BC by promoting tumor progression (Zellmer et al. 2017). As a central messenger, SRC correlated with the regulation of cell growth, differentiation, survival, angiogenesis, and invasion in several important signaling pathways (Espada and Martin-Perez 2017; Yeatman 2004). Highly expressed and activated SRC found in several solid tumors, including breast cancer tissue was involved in promoting cell invasiveness and metastatic potential and decreased the overall survival of patients (Djeungoue-Petga et al. 2019). NOTCH2 regulates tumor growth, invasion and metastasis (Kim et al. 2012; O'Neill et al. 2007). It was found that BC cells with Notch2 knockdown had a slowed down migration and poor cell survival rate, indicating Notch2 may play an oncogenic role (Chao et al. 2014).

To get a deeper insight into the downstream target genes of miR-130b, we analyzed the databases from DIANA-mT, miRDB, miRWalk, Targetscan and GEPIA. 54 common genes were selected, and the PPI network suggested that AR, KIT and ESR1 were hub genes, indicating the pivotal molecular function of miR-130b. The androgen receptor (AR) played a key role in mediating androgens biological effect, that act as a prognostic indicator in tumor growth, metastasis, relapse, hormonal therapy and chemotherapy (Feng et al. 2017). Interestingly, many studies have suggested that the expression level of AR is related to higher prognostic in BC (Qu et al. 2013; Vera-Badillo FE 2014). Proto-oncogene c-Kit (KIT), encodes a receptor tyrosine kinase and participates in regulating cell migration, proliferation and survival (Janostiak et al. 2018). The function of c-KIT in the malignant transformation of tumors might be two faceted, either oncogene or tumor suppressor gene. Recent studies indicated that the malignant transformation of breast epithelium was promoted by downregulating c-KIT expression (Chui 1996; Talaiezadeh et al. 2012), and c-KIT could serve as a biomarker for atypical and malignancy proliferative ductal breast lesions (Janostiak et al. 2018). Estrogen receptor 1 (ESR1) encodes an estrogen receptor that mediates the transcription of many estrogen-inducible genes essential for several reproductive functions, such as cell growth, metabolism, sexual development, and pregnancy, it is also expressed in other non-reproductive tissues such as bone (Bockers et al. 2020; Carleton et al. 2020; Pakharenko et al. 2020). ESR1 mutations have proved to be a key factor in endocrine therapy resistance in BC with hormone receptor-positive. ESR1 mutant provided an enrichment of subclonal cell populations in circulating tumor cells and metastatic sites, which may be partly due to an enhanced "aggressive phenotype" (Dustin et al. 2019). It could be used as a predictive biomarker in hormone-receptor BC, especially in the metastatic setting (Carausu et al. 2019). Based on the finding of current studies, miR-130b might be significantly correlated with BC via the above molecular mechanisms.

In summary, we revealed that the expression level of miR-130b was upregulated in BC tissue compared to normal tissue, which was significantly correlated with a poor prognosis of BC, signify that miR-130b functions as a significant oncogene in BC. Moreover, our study demonstrated that the expression level of miR-130b was regulated by THAP11 and further facilitated BC's development by regulating crucial

downstream genes of the TH signaling pathway, including PFKF, STAT1, SRC, and NOTCH2. On the other hand, we predicted the potential downstream target hub genes of miR-130b, AR, KIT, and ESR1. This study for the first time, reported the novel molecular mechanisms between miR-130b and BC to the best of our knowledge. Therefore, we identified that miR-130b might act as a prognostic biomarker in BC. However, further in-depth research on molecular mechanisms of miR-130b will be helpful to provide a novel therapeutic approach for the treatment of BC.

Conclusion

In conclusion, our study elucidated that miR-130b might be regulated by THAP11 and act as a potential oncogene by mediating the TH signaling pathway and the downstream target hub genes. However, further experiments are still needed to validate the molecular mechanisms of miR-130b in BC.

Declarations

Ethics approval and consent to participate Not applicable

Consent for publication Not applicable

Availability of data and materials The datasets GSE45666

(<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE45666>) and GSE26659

(<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE26659>) analysed during the current study are available in the Gene Expression Omnibus (GEO) database (<http://www.ncbi.nlm.nih.gov/geo/>).

Competing interests The authors declare that they have no conflicts of interest.

Authors' contributions Lijun Hao contributed to the study conception, design and revise the final manuscript critically. Xinyue Chen performed the literature search, data analysis, and written the first draft of the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1 Features of the enrolled datasets

Accession	GPL	Samples		Source
		Control	Breast cancer	
GSE45666	GPL14767	15	101	tissue
GSE26659	GPL8227	17	77	tissue

Table 2 Enriched GO terms and KEGG pathways

Enriched Category	Description	Count	NES	P-Value	FDR
Biological process					
GO:0007210	serotonin receptor signaling pathway	15	2.572	0	0.033
Cellular components					
GO:0043235	receptor complex	118	2.880	0	0
Molecular function					
GO:0030594	neurotransmitter receptor activity	40	3.191	0	0
GO:0042165	neurotransmitter binding	21	2.342	0.002	0.031
GO:0099589	serotonin receptor activity	13	2.333	0.002	0.022
GO:0022803	passive transmembrane transporter activity	114	2.305	0	0.020
GO:0052813	phosphatidylinositol bisphosphate kinase activity	27	2.188	0	0.044
GO:0097472	cyclin-dependent protein kinase activity	20	-2.212	0.002	0.044
GO:0003735	structural constituent of ribosome	25	-2.311	0	0.034
GO:0019840	isoprenoid binding	18	-2.922	0	0.001
KEGG pathway					
hsa04919	Thyroid hormone signaling pathway	36	-2.646	0	0.014
hsa03010	Ribosome	21	-2.467	0.003	0.026
hsa05170	Human immunodeficiency virus 1 infection	47	-2.324	0	0.041

Table 3 Top six genes with high degree of connectivity

Gene symbol	Gene description	Degree
AR	Androgen receptor	9
ESR1	Estrogen receptor 1	7
KIT	KIT proto-oncogene receptor tyrosine kinase	7
ZEB2	Zinc finger E-box binding homeobox 2	6
HSPA8	Heat shock protein family A (Hsp70) member 8	5
MET	MET proto-oncogene, receptor tyrosine kinase	4

Figures

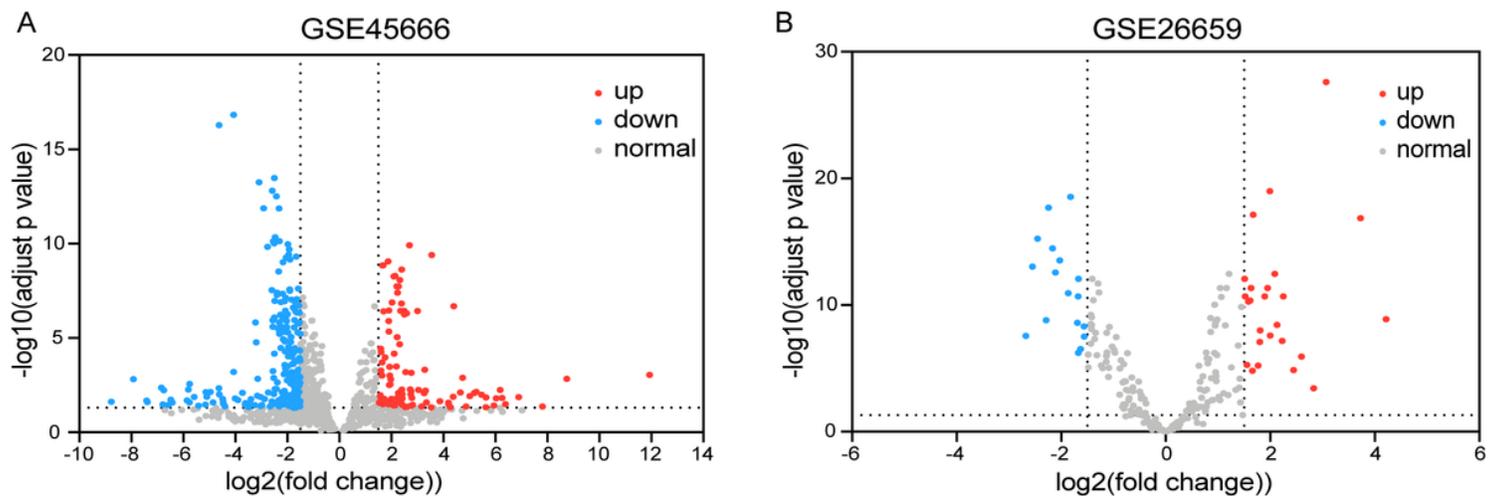
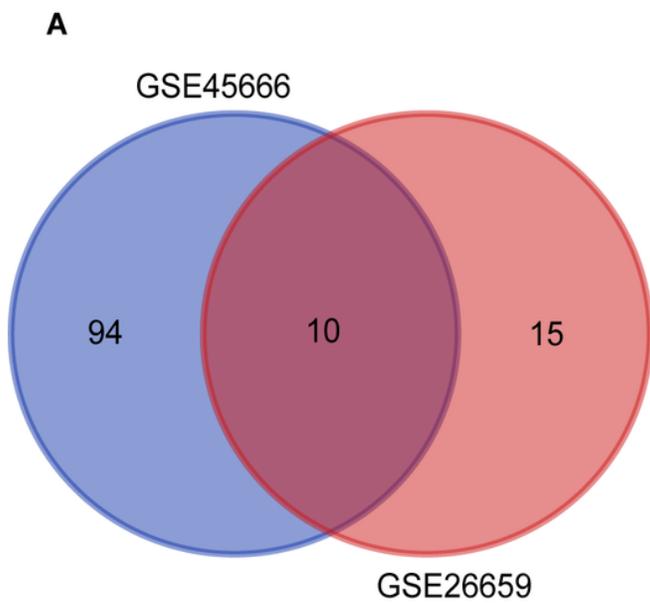


Figure 1

Differentially expressed miRNAs in GSE45666, and GSE26659 a-b Volcano plots show DE-miRNAs between breast cancer tissue and normal breast tissue in two microarray gene profiling datasets. Based on $|\log_2FC \text{ (fold change)}| \geq 1.5$ and adjusted P-value < 0.05 , the red dots imply upregulated miRNAs, and blue dots imply downregulated miRNAs, gray dots means normally expressed miRNAs.



B

DE-miRNAs	GSE45666		GSE26659	
	log ₂ (FC)	adjust p value	log ₂ (FC)	adjust p value
miR-130b	1.656	<0.001	1.582	<0.001
miR-21	1.691	<0.001	3.069	<0.001
miR-425	1.878	<0.001	1.950	<0.001
miR-183	2.277	<0.001	4.215	<0.001
miR-375	1.774	0.020	2.447	<0.001
miR-7	3.006	<0.001	2.829	<0.001
miR-155	2.094	<0.001	2.087	<0.001
miR-210	2.324	<0.001	1.659	<0.001
miR-142-3p	2.356	<0.001	2.129	<0.001
miR-96	2.240	<0.001	3.727	<0.001

Figure 2

Venn diagram of common upregulated DE-miRNAs from GSE45666 and GSE26659 a, Identification of ten DE-miRNAs in two datasets. b, log₂(FC) and adjust p-value of ten DE-miRNAs

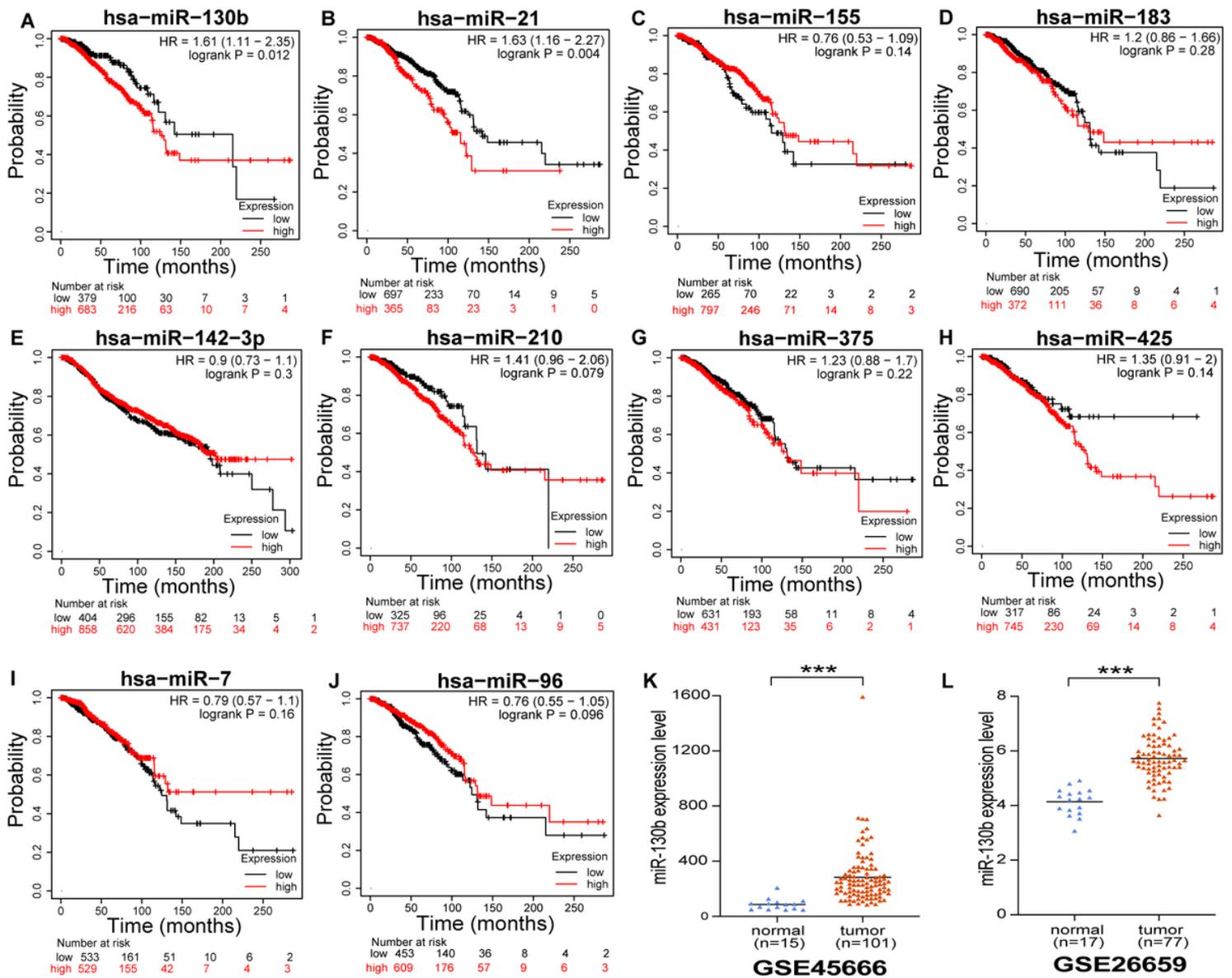


Figure 3

Prognostic value of ten DE-miRNAs in breast cancer based on Kaplan–Meier plot a-b, The expression level of miR-130b and miR-21 was linked to the overall survival of BC ($p < 0.05$) c-j miR-155, miR-183, miR-142-3p, miR-210, miR-375, miR-425, miR-7, miR-96 had no significant difference in revealing prognostic value of BC ($p > 0.05$) (HR, hazard ratio) k-l. The expression level of miR-130b in GSE45666 and GSE26659

A hsa-mir-130b Association Result

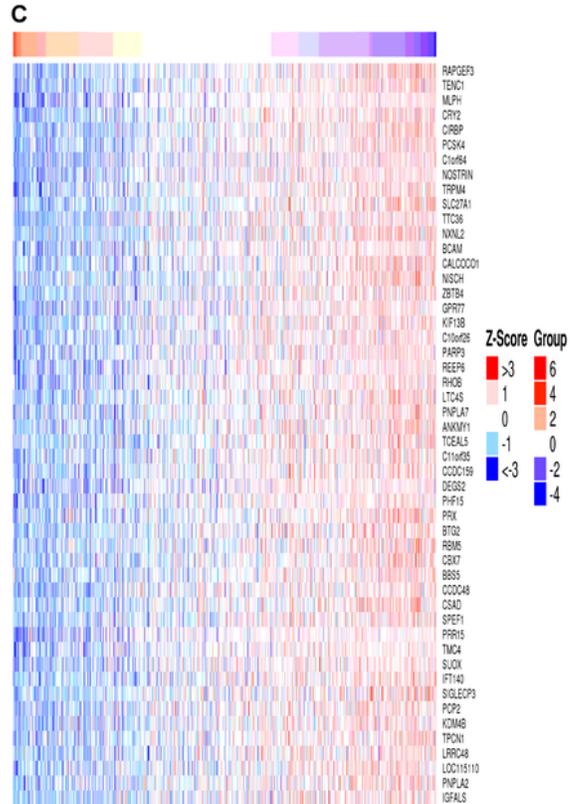
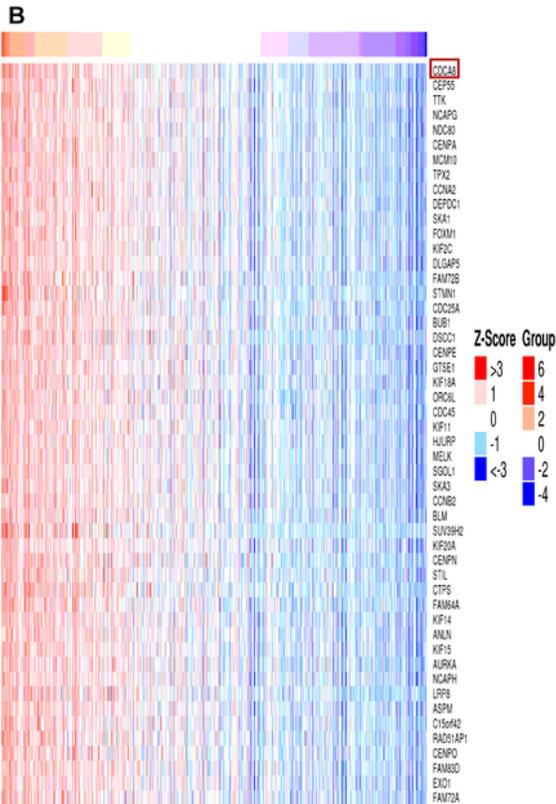
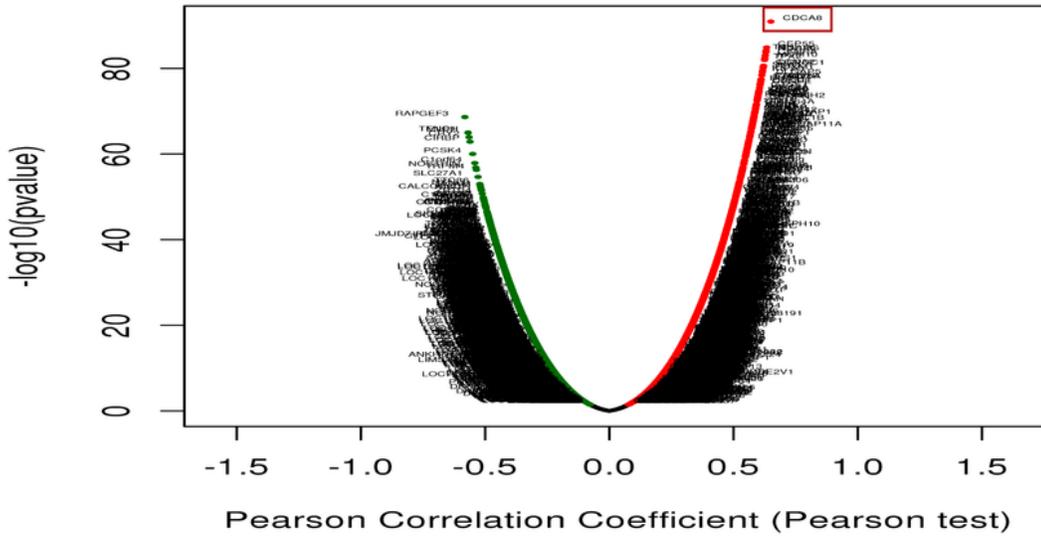


Figure 4

The genes positively and negatively correlated with miR-130b a, An interactive plot revealed miR-130b associated genes in breast cancer. b-c, Heatmap of top 50 positively and negatively correlated significant genes of miR-130b.

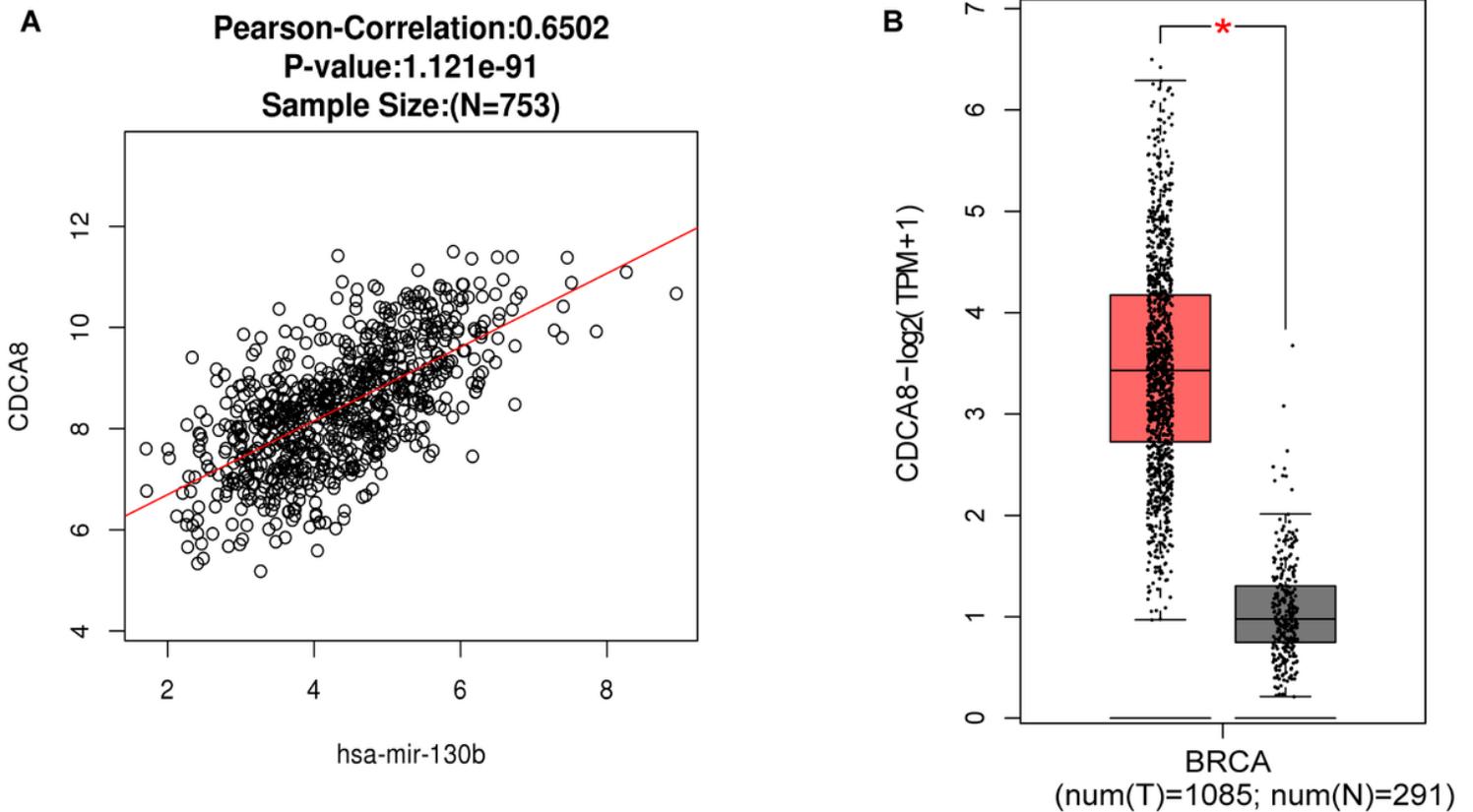


Figure 5

CDCA8 positively associated with miR-130b a, Pearson-correlation between miR-130b and CDCA8, $P < 0.01$. b, The expression level of CDCA8 in BC tissue and normal breast tissue.

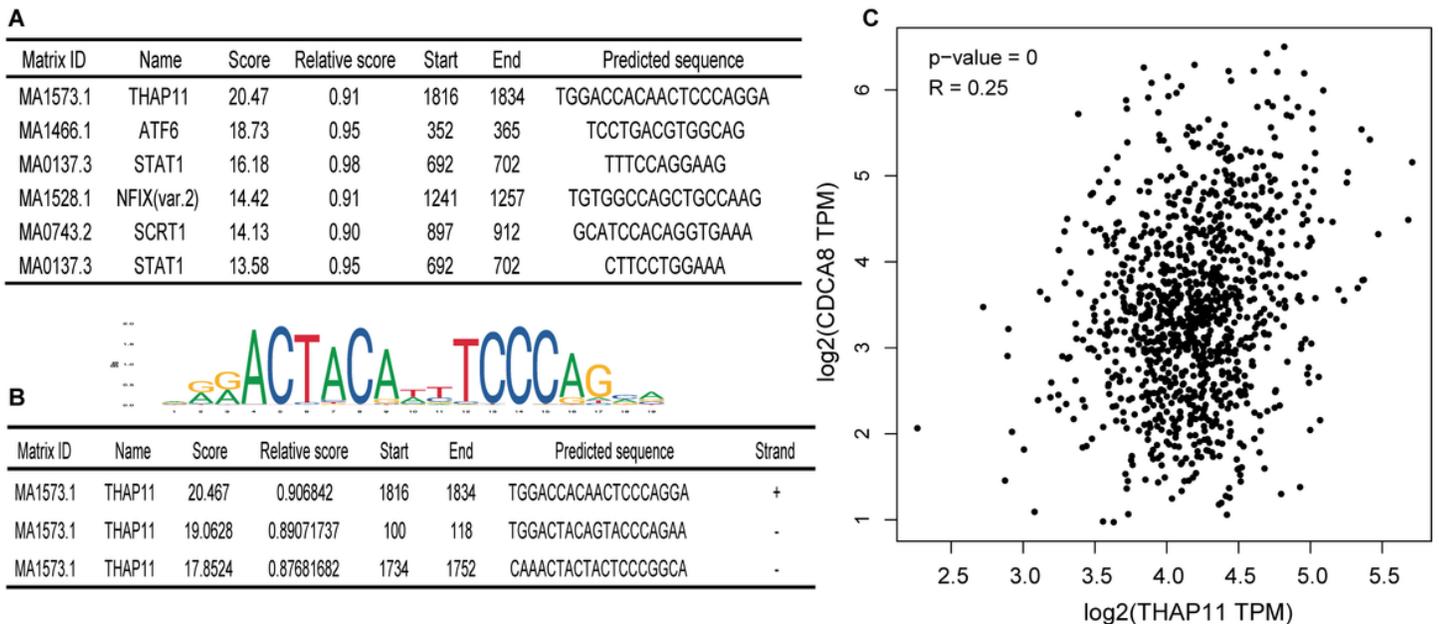


Figure 6

Transcription factor of CDCA8 a, Prediction TF of CDCA8 from JASPAR. b, The relative score and binding site of THAP11. c, Pearson-correlation between THAP11 and CDCA8

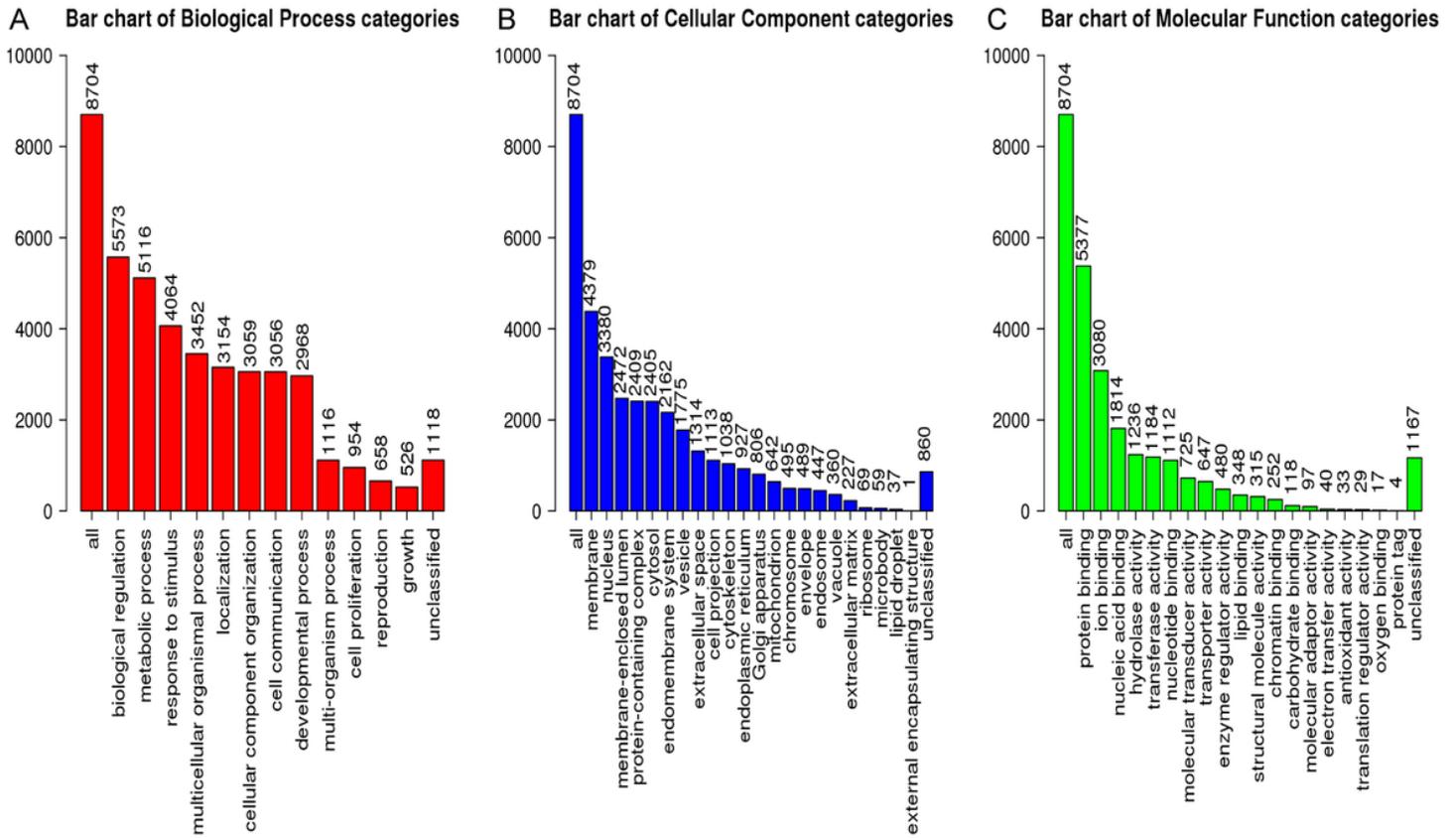


Figure 7

Gene Ontology annotations of genes correlation with miR-130b in BC a, biological process categories. b, cellular component categories c, molecular functions categories

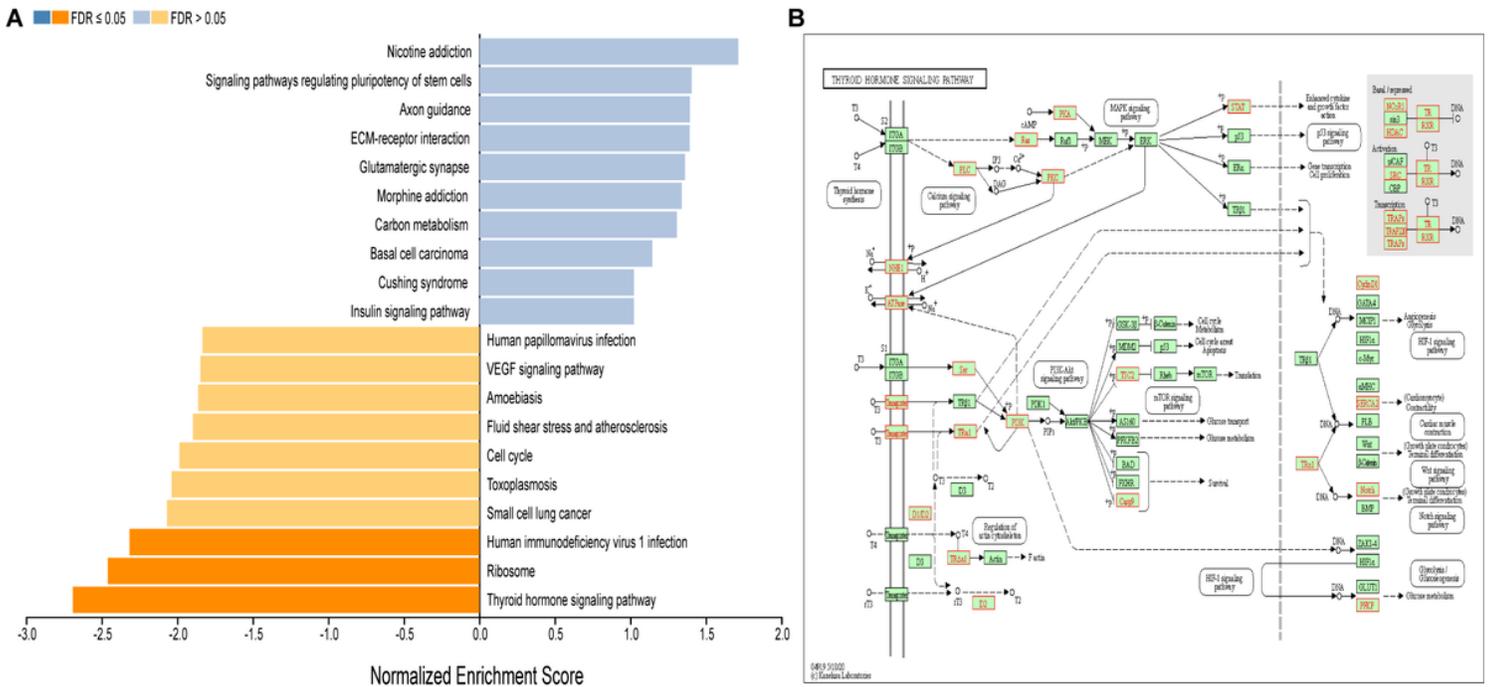


Figure 8

Kyoto Encyclopedia of Genes and Genomes pathway a, the genes correlated with miR-130b were significantly involved in various pathways. b, Thyroid hormone signaling pathway diagram

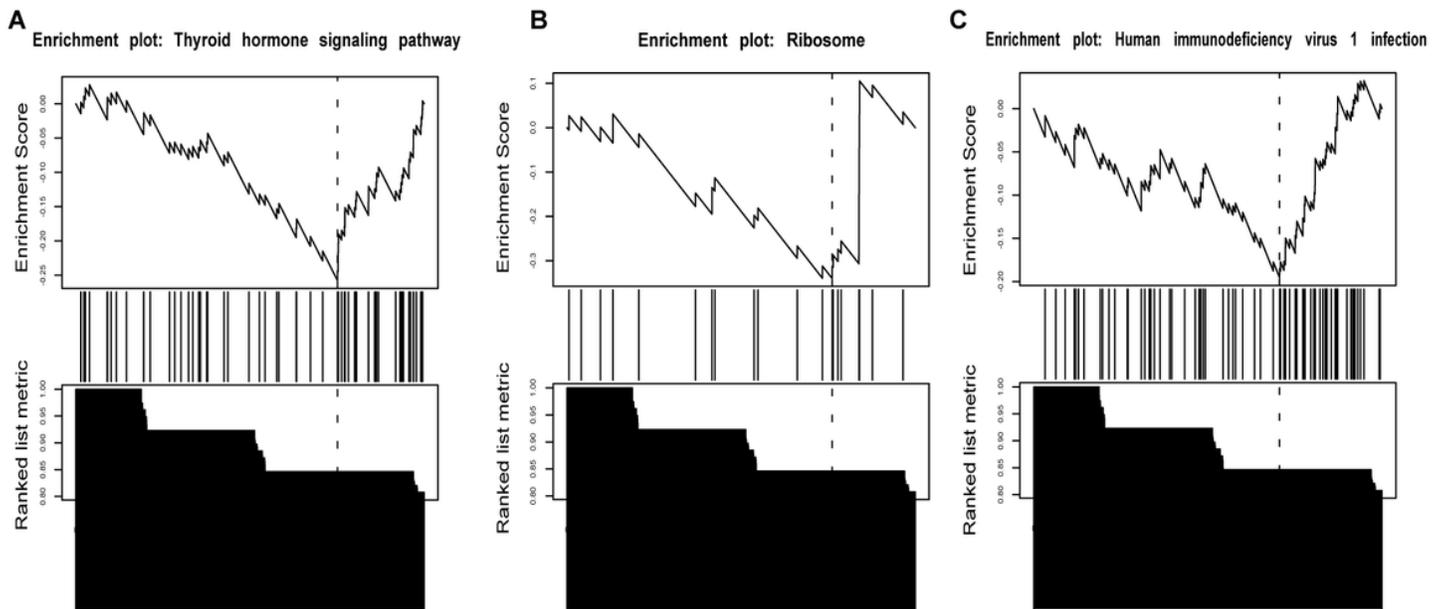


Figure 9

The significant KEGG pathway enrichment plots a, Thyroid hormone signaling pathway, NES=-2.646, FDR=0.014. b, Ribosome, NES=-2.467, FDR=0.026. c, Human immunodeficiency virus 1 infection, NES=-2.324, FDR=0.041

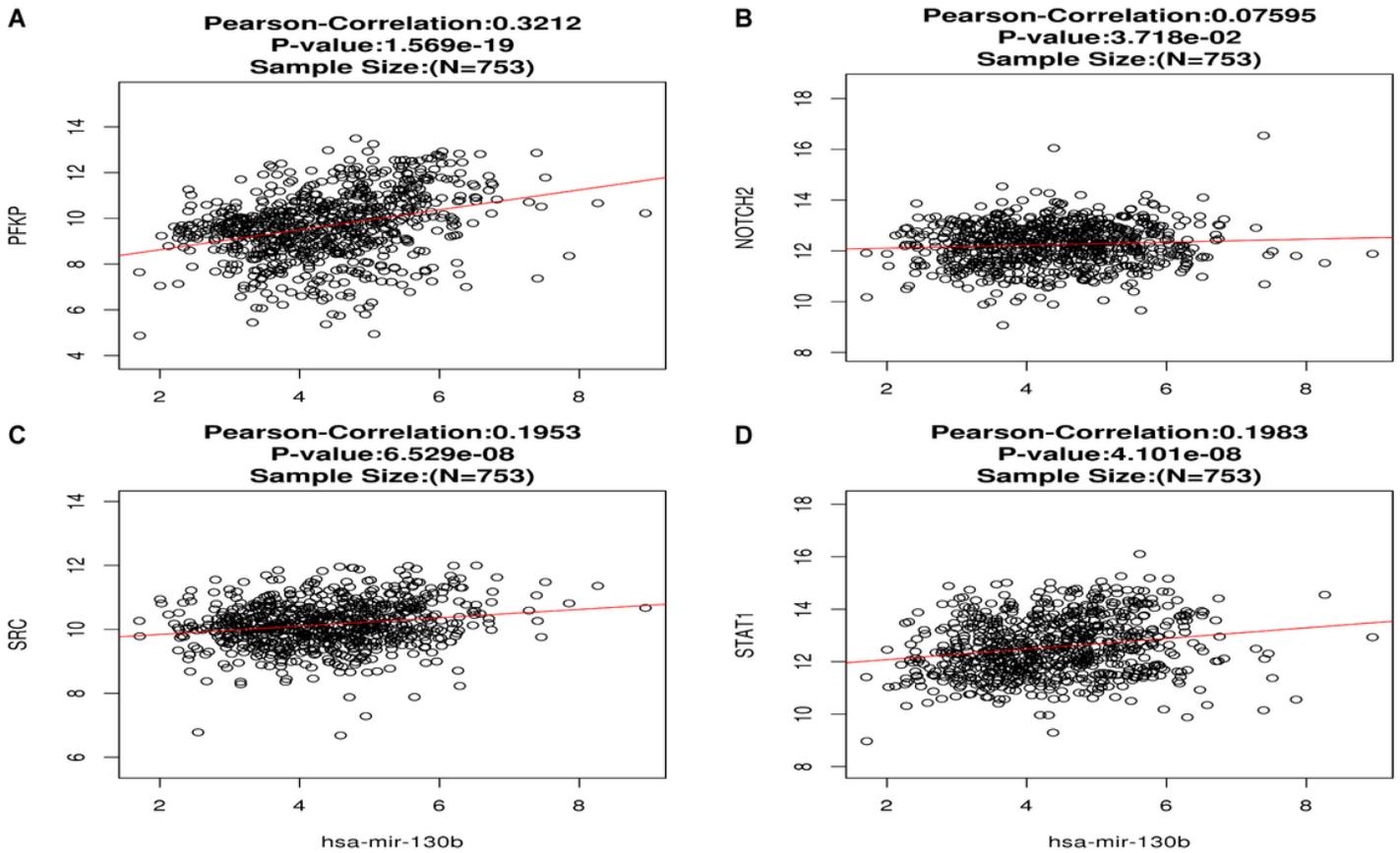


Figure 10

miR-130b positively regulated the genes in the Thyroid hormone signaling pathway a, Pearson-correlation between miR-130b and PFKP, $P < 0.01$. b, Pearson-correlation between miR-130b and NOTCH2, $p=0.037$. c, Pearson-correlation between miR-130b and SRC, $P < 0.01$. d, Pearson-correlation between miR-130b and STAT1, $P < 0.01$

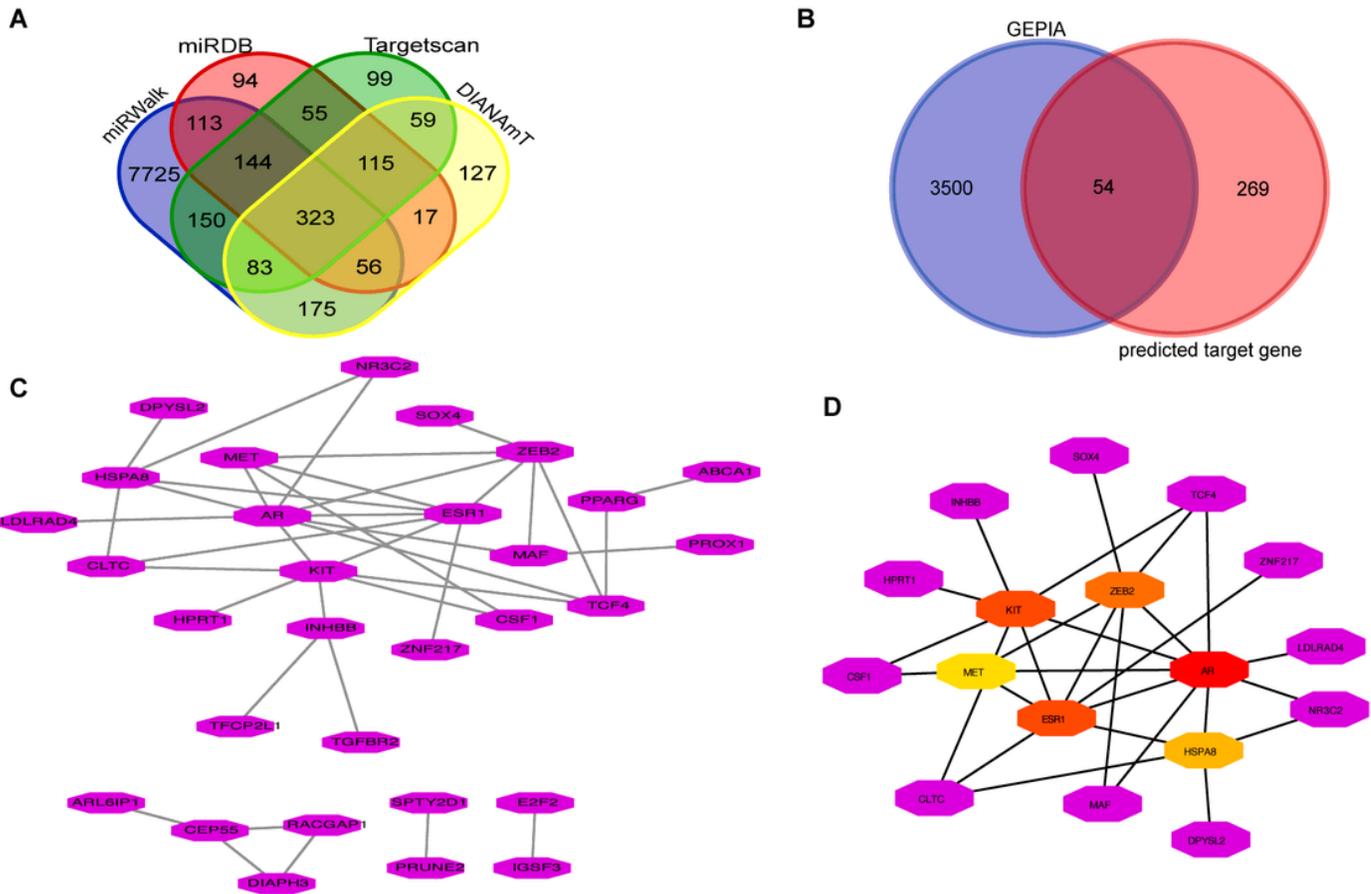


Figure 11

Protein-protein interaction network a, Target genes of miR-130b were acquired from miRWalk, DIANA-mT, miRDB, and Targetscan. b, 54 genes in the overlapping areas of the GEPIA database and target genes. c, PPI network of 54 overlapped genes. d, hub genes in the PPI network, which were defined by the connectivity degree. Red nodes stand for hub genes.