FKBP-related ncRNA-mRNA axis in breast cancer

Hanchu Xiong

Zhejiang University <https://orcid.org/0000-0001-6075-6895>

Zihan Chen

Zhejiang university

Wenwen Zheng

Zhejiang University School of Medicine Second Affiliated Hospital

Jing Sun

Zhejiang University School of Medicine Second Affiliated Hospital

Qingshuang Fu

Ruian City People's Hospital

Rongyue Teng

Zhejiang University School of Medicine Sir Run Run Shaw Hospital

Jida Chen

Zhejiang University School of Medicine Sir Run Run Shaw Hospital

Shuduo Xie

Zhejiang University School of Medicine Sir Run Run Shaw Hospital

Linbo Wang

Zhejiang University

Xiao-Fang Yu

Zhejiang University

Jichun Zhou (✉ jichun-zhou@zju.edu.cn)

<https://orcid.org/0000-0002-0727-4034>

Research article

Keywords: breast cancer; microRNA; long noncoding RNA; FK506-binding protein; bioinformatic analysis.

Posted Date: March 11th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-16732/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Version of Record: A version of this preprint was published at Genomics on November 1st, 2020. See the published version at <https://doi.org/10.1016/j.ygeno.2020.08.017>.

Abstract

Background Breast cancer (BC) is a disease with morbidity ranking the first of women worldwide. FK506-binding protein (FKBP) family has been demonstrated to possess various functions by interacting with different molecular targets in BC. However, a comprehensive ncRNA-mRNA regulatory axis of FKBP has not yet been reported. Methods FKBP related miRNAs were obtained from miRWalk database. Then, potential lncRNAs, transcription factors as well as mRNAs of screened differentially expressed miRNAs (DE-miRNAs) were analysed by using LncBase v.2, miRGen v3 and miRWalk database. Additionally, differential expression and prognostic analysis of lncRNAs were evaluated using TANRIC database. Next, GO annotation and KEGG pathway analysis were processed using DAVID database. Protein-Protein Interaction (PPI) network was established and hub genes were identified using STRING database. Finally, differential expression and prognostic analysis of hub genes were further conducted using UALCAN and bc-GenExMiner v4.2 database, respectively. Results Eleven DE-miRNAs, consisting of four FKBP4 related DE-miRNAs and seven FKBP5 related DE-miRNAs, were screened. 482 predicted lncRNAs were found for DE-miRNAs. Then, expression and prognostic results of nine of top twenty lncRNAs of BC were significantly identified. LINC00662 and LINC00963 expression were significantly associated with patients' overall survival (OS). Then, nine potential upstream transcription factors were identified in motifs of DE-miRNAs. 320 target genes were identified for GO annotation and KEGG pathway analysis, which were mainly enriched in cysteine-type endopeptidase activity involved in apoptotic process. Construction and analysis in PPI network showed that RAB7A was selected as a hub gene with the toppest connectivity scores. Differential expression analysis of nine in top ten hub genes of BC were significantly identified. RAB7A and ARRB1 expression were significantly related with BC patients' OS. Conclusions In current study, we firstly established a predicted FKBP-related ncRNA-mRNA regulatory network, thus exploring a comprehensive interpretation of molecular mechanisms and providing potential clues in seeking novel therapeutics for BC. In the future, much more experiments should be conducted to verify our findings.

Background

Breast cancer (BC) is the most widespread and deadly non-cutaneous tumor in worldwide women[1]. Early detection and comprehensive treatments, which consist of surgery, radiation, chemotherapy, endocrine therapy and targeted therapy, have significantly improved the prognosis in BC patients. However, BC is a heterogeneous disease of various different genetical, pathological, and clinical subtypes[2]. Even though intensive efforts have been made in both basic researches and clinical studies, it's still necessary to find more reliable markers to further improve therapeutics for BC patients.

FK506-binding protein (FKBP) family in Homo sapiens genomes has been found to target on various pathways in embryology, stress reaction, heart function, tumorigenesis and neuronal function[3]. In breast cancer, FKBP4 and FKBP5 are most extensively studied proteins among identified human FKBP, which are demonstrated to interact with Hsp90 to affect steroid hormone receptor function[4]. MicroRNAs (miRNAs) are a cluster of small noncoding RNAs consisting of 20 to 24 nucleotides, regulating targeted gene expression by binding to several selective messenger RNAs (mRNAs) [5]. MiRNAs are also found to exert pivotal roles in the genesis and development of BC. For instance, the miRNA let-7's ability to restrain the expression of metastatic genes could be compromised when long non-coding RNA (lncRNA) H19 expression is upregulated, leading to high expression of c-Myc and activating migration and invasion of BC cells[6]. Despite many researches on miRNA expression and function of BC have been conducted, a comprehensive analysis of FKBP-related ncRNA-mRNA regulatory network via clinical information of BC is still lacking. Construction of predicted ncRNA-mRNA axis contributing to BC might unravel the potential molecular mechanisms underlying processes of miRNAs' impact on BC.

Herein, a total of four FKBP4 related differentially expressed miRNAs (DE-miRNAs) and seven FKBP5 related DE-miRNAs were screened. Subsequently, expression and prognostic analytic result of nine of the top twenty lncRNAs in BC were significantly identified. LINC00662 and LINC00963 expression were significantly associated with patients' overall survival (OS). Then, nine potential upstream transcription factors (TFs) were identified in motifs of DE-miRNAs. 320 target genes were selected for GO annotation and KEGG pathway analysis. Construction of Protein-Protein Interaction (PPI) network showed that RAB7A was recognized as a hub gene with the toppest connectivity scores. Differential expression analysis of nine in top ten hub genes of BC were significantly identified. RAB7A and ARRB1 expression were significantly associated with patients' OS. Finally, a potential FKBP-related ncRNA-mRNA regulatory axis contributing to the onset and development in BC was successfully achieved.

Methods

Verification of FKBP's Expression Levels

The mRNA expressions of twelve FKBP's were further verified using GEPIA, which is a recently developed database for analyzing the RNA sequencing expression data of carcinoma and adjacent samples in the TCGA and the GTEx projects[7].

Screening of Potential miRNAs of FKBP's and Targeted Genes of miRNAs

Both screened miRNAs of FKBP4 and FKBP5 and screened gene targets of miRNAs were conducted using miRWalk database, mainly using for experimentally verified miRNA-target interactions[8], specifically the screened miRNAs and genes were generated by intersection of miRDB and miRTarBase.

Screening Of Potential Lncrnas And Transcription Factors Of Mirnas

Predicted lncRNAs of screened miRNAs were all generated by using LncBase v.2, which is a tool used mainly for discovering the connection between miRNAs and lncRNAs[9]. The upstream TFs of screened miRNAs were analyzed by using miRGen v3, which is mainly conducted for discovering the connection between miRNAs and TFs[10].

Validation Of Lncrna Differential Expressions And Prognostic Functions

Both expression levels of top twenty lncRNAs in different subtypes and their prognostic roles of overall survival of BC patients were further validated by using The Atlas of ncRNA in Cancer (TANRIC), an open-access database for interactive exploration of lncRNAs of various cancer[11]. lncRNAs with $|\log_2FC| > 2$ and $P < 0.05$ were regarded as statistically significant.

Go Annotation And Kegg Pathway Analysis

To better understand those screened candidate targeted genes, DAVID database was used to perform GO annotation and KEGG pathway analysis. The top ten enriched GO items were all listed in Fig. S4A-S4C. BP analysis revealed that candidate targeted genes of screened DE-miRNAs were significantly enriched in negative regulation of cysteine-type endopeptidase activity involved in apoptotic process (Fig. S4A). As for CC analysis, genes were significantly enriched in protein complex, postsynaptic density and cytoplasmic vesicle membrane (Fig. S4B). MF analysis for these genes consisted of protein binding, zinc ion binding and cadherin binding involved in cell-cell adhesion (Fig. S4C). KEGG pathway analysis was further utilized for candidate targeted genes of screened DE-miRNAs. As shown in Table 7, candidate targeted genes of screened DE-miRNAs were markedly enriched in Axon guidance.

Table 7
The KEGG pathway analysis of DE-miRNAs related targeted genes

Term	Count	Genes	PValue	Benjamini	FDR
hsa04360:Axon guidance	6	ABLIM1, SEMA4G, PLXNA2, EFNA3, NTN1, EPHB2	0.02951	0.991709556	30.3503
hsa04971:Gastric acid secretion	4	ATP1B4, CALM3, SLC4A2, PLCB1	0.077583	0.998436226	62.28624
hsa04152:AMPK signaling pathway	5	PDPK1, SLC2A4, PFKFB3, SCD, PPP2R5E	0.087221	0.992305724	66.77845
hsa04960:Aldosterone-regulated sodium reabsorption	3	SGK1, PDPK1, ATP1B4	0.098903	0.984480772	71.56362

Establishment Of Protein-protein Interaction Network

To better understanding the relationship among targeted genes of miRNAs, the PPI network was established by using the STRING database[13]. PPI node pairs with the score > 0.4 were chosen for further analysis. The top ten hub genes were verified based on the node number in the PPI network.

Verification Of Hub Gene Differential Expressions

The mRNA expressions of hub genes in BC were further verified by using UALCAN (<http://ualcan.path.uab.edu>), which is an interactive online database to perform in-depth analyses of gene expression data from TCGA[14].

Verification Of Hub Gene Prognostic Roles

The prognostic results of potential hub genes in BC were analyzed by using bc-GenExMiner v4.2 (bcgenex.centregauducheau.fr), a statistical mining tool of published BC transcriptomic data from TCGA and GEO[15].

Statistical Analysis

Majority of the statistical analysis was done through the above-mentioned bioinformatic tools, and only lncRNAs or miRNAs or genes with $|\log_2FC| > 2$ and $P < 0.05$ were regarded as statistically significant.

Results

Validation of Candidate DE-miRNAs

Firstly, GEPIA database was utilized to detect gene expressions of twelve FKBP family members. As shown in Fig. S1E and S1F, expression level of FKBP4 was significantly higher in BC tissues than that in adjacent tissues, while FKBP5 expression was significantly lower in BC tissues than that in

adjacent tissues. Differential expression analysis of other genes showed no significant changes between BC and normal tissues (Fig. S1A-S1D and S1G-S1L). Then, to validate potential FKBP mRNA-miRNA regulatory axis in BC, miRWalk database was used to screen differentially expressed miRNAs of both BC samples and adjacent samples. As shown in Fig. 1A and 1B, eleven significantly DE-miRNAs (hsa-miR-423-5p, hsa-miR-3202, hsa-miR-4519, hsa-miR-6750-5p, hsa-miR-4740-5p, hsa-miR-4779, hsa-miR-377-5p, hsa-miR-510-5p, hsa-miR-3613-3p, hsa-miR-6086 and hsa-miR-7106-5p) were finally identified by intersection of miRDB and miRTarBase. These predicted target DE-miRNAs were also listed in Tables 1 and 2.

Table 1
The predicted targeted DE-miRNAs of FKBP4

miRNA	RefseqID	GeneSymbol	Score	Position	Binding Site	Au	Me	N Pairings	Targetscan	Mirdb	Mirtarbase
hsa-miR-423-5p	NM_002014	FKBP4	1	3UTR	20,412,060	0.48	-4.529	17	LINK	Link	MIRT038093
hsa-miR-423-5p	NM_002014	FKBP4	1	3UTR	28,792,901	0.37	-11.667	17	LINK	Link	MIRT038093
hsa-miR-3202	NM_002014	FKBP4	1	3UTR	29,572,977	0.46	-7.861	15	—	Link	MIRT741143
hsa-miR-3202	NM_002014	FKBP4	1	3UTR	20,472,062	0.47	-6.851	13	—	Link	MIRT741143
hsa-miR-4519	NM_002014	FKBP4	1	3UTR	24,172,451	0.4	-6.2	14	—	Link	MIRT745785
hsa-miR-6750-5p	NM_002014	FKBP4	1	3UTR	21,022,125	0.46	-10.109	20	—	Link	MIRT754571

Table 2
The predicted targeted DE-miRNAs of FKBP5

miRNA	RefseqID	GeneSymbol	Score	Position	Binding Site	Au	Me	N Pairings	Targetscan	Mirdb	Mirtarbase
hsa-miR-4740-5p	NM_001145775	FKBP5	1	3UTR	18,541,869	0.34	-16.423	14	—	Link	MIRT537338
hsa-miR-4779	NM_001145775	FKBP5	1	3UTR	30,823,098	0.56	-8.34	13	—	Link	MIRT452010
hsa-miR-4740-5p	NM_004117	FKBP5	1	3UTR	16,961,711	0.34	-16.423	14	—	Link	MIRT537338
hsa-miR-4779	NM_004117	FKBP5	1	3UTR	29,292,940	0.56	-8.34	10	—	Link	MIRT452010
hsa-miR-4740-5p	NM_001145776	FKBP5	1	3UTR	17,411,756	0.34	-16.423	14	—	Link	MIRT537338
hsa-miR-4779	NM_001145776	FKBP5	1	3UTR	29,742,985	0.56	-8.34	10	—	Link	MIRT452010
hsa-miR-377-5p	NM_001145777	FKBP5	0.96	3UTR	58,765,908	0.53	-10.729	14	—	Link	MIRT451979
hsa-miR-377-5p	NM_001145777	FKBP5	1	3UTR	12,801,301	0.73	-8.769	19	—	Link	MIRT451979
hsa-miR-510-5p	NM_001145777	FKBP5	1	3UTR	35,733,613	0.59	-5.227	15	—	Link	MIRT514719
hsa-miR-3613-3p	NM_001145777	FKBP5	1	3UTR	55,875,601	0.53	-21.723	11	—	Link	MIRT765989
hsa-miR-6086	NM_001145777	FKBP5	1	3UTR	64,156,458	0.46	-3.938	20	—	Link	MIRT451978
hsa-miR-6086	NM_001145777	FKBP5	1	3UTR	47,484,765	0.56	-5.694	16	—	Link	MIRT451978
hsa-miR-7106-5p	NM_001145777	FKBP5	1	3UTR	13,441,367	0.54	-3.938	18	—	Link	MIRT451992
hsa-miR-7106-5p	NM_001145777	FKBP5	1	3UTR	32,213,240	0.6	-8.574	18	—	Link	MIRT451992

Prediction Of De-mirnas Associated Lncrnas

Here, we first intended to identify candidate lncRNAs of DE-miRNAs by using LncBase v.2 database. The 20 most frequent lncRNAs (LINC00662, LRRc75A-AS1, LINC01002, KCNQ1OT1, RP11-15H20.6, ZNF213-AS1, LINC00963, AC007246.3, XLOC_013274, XIST, ERVK3-1, AC138035.2, RP11-34P13.13, LINC00960, FAM211A-AS1, AC013394.2, AC005154.6, ZNF561-AS1, XLOC_014159, XLOC_009145) for screened DE-miRNAs were presented in Fig. 1C. These predicted target DE-miRNAs associated lncRNAs were also listed in Table 3.

Table 3
The predicted targeted DE-miRNAs associated
lncRNAs

lncRNA	miRNA
UCA1	hsa-miR-423-5p
KCNQ10T1	hsa-miR-423-5p
FOXP4-AS1	hsa-miR-423-5p
AC068039.4	hsa-miR-423-5p
SNORA67	hsa-miR-423-5p
MALAT1	hsa-miR-423-5p
XLOC_014255	hsa-miR-423-5p
SPACA6P	hsa-miR-423-5p
AC004951.6	hsa-miR-423-5p
KCNQ10T1	hsa-miR-3202
RP11-20D14.6	hsa-miR-3202
XIST	hsa-miR-3202
AC093642.3	hsa-miR-3202
XLOC_011789	hsa-miR-3202
LOC648987	hsa-miR-3202
GMDS-AS1	hsa-miR-3202
RP5-1085F17.3	hsa-miR-3202
LOC100190986	hsa-miR-3202
SNHG8	hsa-miR-3202
LINC00960	hsa-miR-3202
AC007255.8	hsa-miR-3202
APTR	hsa-miR-3202
XLOC_014159	hsa-miR-3202
APTR	hsa-miR-3202
APTR	hsa-miR-3202
RP11-140K17.3	hsa-miR-3202
GMDS-AS1	hsa-miR-3202
AC005154.6	hsa-miR-3202
KTN1-AS1	hsa-miR-3202
RP11-513I15.6	hsa-miR-3202
PCBP1-AS1	hsa-miR-3202
MIR503HG	hsa-miR-3202
AC007246.3	hsa-miR-3202
XXbac-BPG154L12.4	hsa-miR-3202
FAM201A	hsa-miR-3202
ZEB1-AS1	hsa-miR-3202
AC007246.3	hsa-miR-3202
TINCR	hsa-miR-3202

lncRNA	miRNA
XLOC_010706	hsa-miR-3202
TINCR	hsa-miR-3202
AC007246.3	hsa-miR-3202
LINC01278	hsa-miR-3202
SLC25A25-AS1	hsa-miR-3202
RP11-388C12.8	hsa-miR-3202
AC007246.3	hsa-miR-3202
RP11-734K2.4	hsa-miR-3202
CTD-2284J15.1	hsa-miR-3202
MGC27345	hsa-miR-3202
AC007246.3	hsa-miR-3202
RP11-458F8.4	hsa-miR-3202
RP11-395P17.3	hsa-miR-3202
ZEB1-AS1	hsa-miR-3202
LINC00960	hsa-miR-3202
RP11-725P16.2	hsa-miR-3202
AC007246.3	hsa-miR-3202
MIR4697HG	hsa-miR-3202
RP11-545I5.3	hsa-miR-3202
RP11-197N18.2	hsa-miR-3202
XLOC_006242	hsa-miR-3202
KCNQ1OT1	hsa-miR-4519
RP11-539L10.3	hsa-miR-4519
RP11-539L10.3	hsa-miR-4519
XLOC_003870	hsa-miR-4519
CTC-459F4.3	hsa-miR-4519
LINC00663	hsa-miR-4519
AC025171.1	hsa-miR-4519
XLOC_013499	hsa-miR-4519
LINC00662	hsa-miR-4519
RP11-574K11.29	hsa-miR-4519
HNRNPU-AS1	hsa-miR-4519
RP11-932O9.9	hsa-miR-4519
XLOC_011248	hsa-miR-4519
RP11-182L21.6	hsa-miR-4519
ERVK3-1	hsa-miR-6750-5p
XLOC_009783	hsa-miR-6750-5p
KCNQ1OT1	hsa-miR-6750-5p
CTD-2006C1.2	hsa-miR-6750-5p
CASC2	hsa-miR-6750-5p
LINC00960	hsa-miR-6750-5p

lncRNA	miRNA
ERVK3-1	hsa-miR-6750-5p
CTC-241N9.1	hsa-miR-6750-5p
CTA-392E5.1	hsa-miR-6750-5p
ZNF213-AS1	hsa-miR-6750-5p
NDUFA6-AS1	hsa-miR-6750-5p
NEAT1	hsa-miR-6750-5p
RP5-1065J22.8	hsa-miR-6750-5p
GMDS-AS1	hsa-miR-6750-5p
ZNF213-AS1	hsa-miR-6750-5p
ERVK3-1	hsa-miR-6750-5p
C1QTNF9B-AS1	hsa-miR-6750-5p
LINC00662	hsa-miR-4740-5p
UCA1	hsa-miR-4740-5p
CTD-2017D11.1	hsa-miR-4740-5p
LOC100506639	hsa-miR-4740-5p
RP11-658F2.8	hsa-miR-4740-5p
RP11-440L14.1	hsa-miR-4740-5p
A1BG-AS1	hsa-miR-4740-5p
A1BG-AS1	hsa-miR-4740-5p
CTD-2017D11.1	hsa-miR-4740-5p
ARHGEF26-AS1	hsa-miR-4740-5p
XIST	hsa-miR-4740-5p
RP4-806M20.3	hsa-miR-4740-5p
XIST	hsa-miR-4740-5p
KCNQ10T1	hsa-miR-4740-5p
RP5-890E16.2	hsa-miR-4740-5p
TMPO-AS1	hsa-miR-4740-5p
TMPO-AS1	hsa-miR-4740-5p
XLOC_006476	hsa-miR-4779
RP11-10L12.4	hsa-miR-4779
KCNQ10T1	hsa-miR-4779
LINC00662	hsa-miR-4779
LINC00662	hsa-miR-4779
AC013394.2	hsa-miR-4779
RP11-111F5.4	hsa-miR-4779
AC013394.2	hsa-miR-4779
AC013394.2	hsa-miR-4779
CTC-459F4.3	hsa-miR-4779
RP11-111F5.4	hsa-miR-4779
GLIDR	hsa-miR-4779
AC005154.6	hsa-miR-4779

lncRNA	miRNA
XLOC_007690	hsa-miR-4779
LINC01278	hsa-miR-4779
LINC00925	hsa-miR-4779
SH3BP5-AS1	hsa-miR-4779
RP11-111F5.4	hsa-miR-4779
RP11-440L14.1	hsa-miR-4779
LINC00925	hsa-miR-4779
RP11-440L14.1	hsa-miR-4779
IQCH-AS1	hsa-miR-4779
AC005154.6	hsa-miR-4779
IQCH-AS1	hsa-miR-4779
LOC100129917	hsa-miR-4779
CASC2	hsa-miR-4779
LBX2-AS1	hsa-miR-4779
LOC100190986	hsa-miR-4779
RP4-773N10.4	hsa-miR-4779
AC062029.1	hsa-miR-4779
XLOC_014159	hsa-miR-4779
LINC00680	hsa-miR-4779
LINC00662	hsa-miR-377-5p
LINC00662	hsa-miR-377-5p
LINC00662	hsa-miR-377-5p
KCNQ10T1	hsa-miR-377-5p
ZNF213-AS1	hsa-miR-377-5p
CTC-559E9.6	hsa-miR-377-5p
XLOC_013274	hsa-miR-377-5p
AC138035.2	hsa-miR-377-5p
XLOC_006058	hsa-miR-377-5p
RP11-15H20.6	hsa-miR-377-5p
CTBP1-AS2	hsa-miR-377-5p
LINC00662	hsa-miR-377-5p
CTC-273B12.8	hsa-miR-377-5p
LINC00662	hsa-miR-377-5p
XLOC_012370	hsa-miR-377-5p
LOC100288069	hsa-miR-377-5p
LINC00265	hsa-miR-510-5p
LINC01347	hsa-miR-510-5p
LINC01002	hsa-miR-510-5p
XLOC_013274	hsa-miR-510-5p
LOC284581	hsa-miR-510-5p
KCNQ10T1	hsa-miR-510-5p

lncRNA	miRNA
FAM157C	hsa-miR-510-5p
LOC284581	hsa-miR-510-5p
XLOC_007970	hsa-miR-510-5p
XLOC_009145	hsa-miR-510-5p
RP11-457M11.5	hsa-miR-510-5p
RP5-1092A3.4	hsa-miR-510-5p
XLOC_012427	hsa-miR-510-5p
LINC01420	hsa-miR-510-5p
RP11-1149O23.3	hsa-miR-510-5p
LINC00662	hsa-miR-510-5p
RP5-890E16.2	hsa-miR-510-5p
ZNF674-AS1	hsa-miR-510-5p
RP11-712L6.5	hsa-miR-510-5p
SNHG3	hsa-miR-510-5p
MKLN1-AS	hsa-miR-510-5p
GS1-358P8.4	hsa-miR-510-5p
KB-1507C5.2	hsa-miR-510-5p
RP11-477H21.2	hsa-miR-510-5p
RP11-434D9.1	hsa-miR-510-5p
STAG3L5P-PVRIG2P-PILRB	hsa-miR-510-5p
RP11-644F5.11	hsa-miR-510-5p
STAG3L5P-PVRIG2P-PILRB	hsa-miR-510-5p
FLJ37035	hsa-miR-510-5p
RP11-473M20.9	hsa-miR-510-5p
KB-1507C5.2	hsa-miR-510-5p
XLOC_001223	hsa-miR-510-5p
XLOC_013274	hsa-miR-3613-3p
KCNQ10T1	hsa-miR-3613-3p
LINC00662	hsa-miR-3613-3p
LINC00662	hsa-miR-3613-3p
XLOC_006242	hsa-miR-3613-3p
LINC01002	hsa-miR-3613-3p
RP11-34P13.13	hsa-miR-3613-3p
ZNF561-AS1	hsa-miR-3613-3p
RP11-15H20.6	hsa-miR-3613-3p
CTB-89H12.4	hsa-miR-3613-3p
CEBPZ-AS1	hsa-miR-3613-3p
RP11-15H20.6	hsa-miR-3613-3p
CEBPZ-AS1	hsa-miR-3613-3p
RP1-283E3.8	hsa-miR-3613-3p
RP11-498C9.15	hsa-miR-3613-3p

lncRNA	miRNA
XIST	hsa-miR-3613-3p
GABPB1-AS1	hsa-miR-3613-3p
HCG11	hsa-miR-3613-3p
RP11-156E6.1	hsa-miR-3613-3p
TUG1	hsa-miR-3613-3p
CTD-2574D22.4	hsa-miR-3613-3p
SNHG16	hsa-miR-3613-3p
XLOC_006058	hsa-miR-3613-3p
CTD-3220F14.1	hsa-miR-3613-3p
RP11-34P13.13	hsa-miR-3613-3p
LOC100506730	hsa-miR-3613-3p
LINC00963	hsa-miR-3613-3p
RP5-1085F17.3	hsa-miR-3613-3p
FGD5-AS1	hsa-miR-3613-3p
RP11-159D12.2	hsa-miR-3613-3p
LINC01087	hsa-miR-3613-3p
LRR75A-AS1	hsa-miR-3613-3p
RP11-182L21.6	hsa-miR-3613-3p
CASC7	hsa-miR-3613-3p
CTC-444N24.11	hsa-miR-3613-3p
RP11-115C21.2	hsa-miR-3613-3p
NUTM2B-AS1	hsa-miR-3613-3p
LOC100190986	hsa-miR-3613-3p
LINC00963	hsa-miR-3613-3p
FAM201A	hsa-miR-3613-3p
TUG1	hsa-miR-3613-3p
RPARP-AS1	hsa-miR-3613-3p
AF127936.7	hsa-miR-3613-3p
AC025335.1	hsa-miR-3613-3p
LINC00963	hsa-miR-3613-3p
BCYRN1	hsa-miR-3613-3p
LRR75A-AS1	hsa-miR-3613-3p
LOC284023	hsa-miR-3613-3p
XLOC_001417	hsa-miR-3613-3p
RP11-747H7.3	hsa-miR-3613-3p
RP11-705C15.3	hsa-miR-3613-3p
CTD-2044J15.2	hsa-miR-3613-3p
CTD-3074O7.12	hsa-miR-3613-3p
AC159540.1	hsa-miR-3613-3p
OIP5-AS1	hsa-miR-3613-3p
LINC00342	hsa-miR-3613-3p

lncRNA	miRNA
CTD-3092A11.2	hsa-miR-3613-3p
LINC00338	hsa-miR-3613-3p
RP11-469M7.1	hsa-miR-3613-3p
U91328.19	hsa-miR-3613-3p
TUG1	hsa-miR-3613-3p
MGC27345	hsa-miR-3613-3p
XLOC_000441	hsa-miR-3613-3p
LRR75A-AS1	hsa-miR-3613-3p
CTD-2369P2.2	hsa-miR-3613-3p
LRR75A-AS1	hsa-miR-3613-3p
FLJ31306	hsa-miR-3613-3p
KB-1460A1.5	hsa-miR-3613-3p
LINC00680	hsa-miR-3613-3p
ZNF213-AS1	hsa-miR-3613-3p
LINC01355	hsa-miR-3613-3p
LINC01002	hsa-miR-3613-3p
GLG1	hsa-miR-3613-3p
SNHG20	hsa-miR-3613-3p
RP11-513I15.6	hsa-miR-3613-3p
RP11-46C24.7	hsa-miR-3613-3p
LINC00938	hsa-miR-3613-3p
FAM157C	hsa-miR-3613-3p
LOC648987	hsa-miR-3613-3p
AC083843.1	hsa-miR-3613-3p
NEAT1	hsa-miR-3613-3p
LRR75A-AS1	hsa-miR-3613-3p
RP11-361F15.2	hsa-miR-3613-3p
COX10-AS1	hsa-miR-3613-3p
RP5-1074L1.4	hsa-miR-3613-3p
RP5-1092A3.4	hsa-miR-3613-3p
CTD-3252C9.4	hsa-miR-3613-3p
LINC00657	hsa-miR-3613-3p
RP11-395B7.7	hsa-miR-3613-3p
RP11-798M19.6	hsa-miR-3613-3p
LINC00467	hsa-miR-3613-3p
RP11-206L10.5	hsa-miR-3613-3p
SNHG16	hsa-miR-3613-3p
XLOC_003416	hsa-miR-3613-3p
XLOC_007970	hsa-miR-3613-3p
RP11-6N17.4	hsa-miR-3613-3p
NUTM2B-AS1	hsa-miR-3613-3p

lncRNA	miRNA
FLJ10038	hsa-miR-3613-3p
LINC00467	hsa-miR-3613-3p
RP11-159D12.2	hsa-miR-3613-3p
XLOC_010212	hsa-miR-3613-3p
LRRC75A-AS1	hsa-miR-3613-3p
LRRC75A-AS1	hsa-miR-3613-3p
LINC00662	hsa-miR-3613-3p
CTC-273B12.8	hsa-miR-3613-3p
CTC-365E16.1	hsa-miR-3613-3p
AC005154.6	hsa-miR-3613-3p
ZNF674-AS1	hsa-miR-3613-3p
FAM211A-AS1	hsa-miR-3613-3p
XLOC_008461	hsa-miR-3613-3p
LRRC75A-AS1	hsa-miR-3613-3p
RP11-395P17.3	hsa-miR-3613-3p
LINC00963	hsa-miR-3613-3p
LRRC75A-AS1	hsa-miR-3613-3p
LRRC75A-AS1	hsa-miR-3613-3p
MIR17HG	hsa-miR-3613-3p
LINC00630	hsa-miR-3613-3p
PCAT7	hsa-miR-3613-3p
LRRC75A-AS1	hsa-miR-3613-3p
RP11-282O18.3	hsa-miR-3613-3p
RP11-350F4.2	hsa-miR-3613-3p
CTC-273B12.8	hsa-miR-3613-3p
SLC25A25-AS1	hsa-miR-3613-3p
SNHG16	hsa-miR-3613-3p
AC013394.2	hsa-miR-3613-3p
PCBP2-OT1	hsa-miR-3613-3p
AC011747.4	hsa-miR-3613-3p
LINC00662	hsa-miR-3613-3p
FAM211A-AS1	hsa-miR-3613-3p
RP11-159D12.2	hsa-miR-3613-3p
RP11-174G6.5	hsa-miR-3613-3p
LRRC75A-AS1	hsa-miR-3613-3p
FAM211A-AS1	hsa-miR-3613-3p
SNHG3	hsa-miR-3613-3p
FAM211A-AS1	hsa-miR-3613-3p
LINC01125	hsa-miR-3613-3p
RP11-798M19.6	hsa-miR-3613-3p
MIR17HG	hsa-miR-3613-3p

lncRNA	miRNA
AC016747.3	hsa-miR-3613-3p
PDXDC2P	hsa-miR-3613-3p
RP11-226L15.5	hsa-miR-3613-3p
AC005562.1	hsa-miR-3613-3p
ZNF213-AS1	hsa-miR-3613-3p
XLOC_009145	hsa-miR-3613-3p
LINC00052	hsa-miR-3613-3p
TMEM191C	hsa-miR-3613-3p
RP11-443B7.1	hsa-miR-3613-3p
LRRC75A-AS1	hsa-miR-3613-3p
LINC01347	hsa-miR-3613-3p
XLOC_011248	hsa-miR-3613-3p
CKMT2-AS1	hsa-miR-3613-3p
LINC00662	hsa-miR-6086
LINC00662	hsa-miR-6086
LINC00662	hsa-miR-6086
KCNQ10T1	hsa-miR-6086
RP11-15H20.6	hsa-miR-6086
XLOC_013274	hsa-miR-6086
CTC-559E9.6	hsa-miR-6086
LINC00662	hsa-miR-6086
LINC00662	hsa-miR-6086
CTC-260E6.6	hsa-miR-6086
XLOC_012370	hsa-miR-6086
ERVK3-1	hsa-miR-6086
LOC100129034	hsa-miR-6086
ZNF213-AS1	hsa-miR-6086
AC138035.2	hsa-miR-6086
RP11-15E18.1	hsa-miR-6086
RP11-212P7.2	hsa-miR-6086
TTN-AS1	hsa-miR-6086
RP11-34P13.13	hsa-miR-7106-5p
LOC100190986	hsa-miR-7106-5p
KCNQ10T1	hsa-miR-7106-5p
ZNF561-AS1	hsa-miR-7106-5p
LINC00174	hsa-miR-7106-5p
AC138035.2	hsa-miR-7106-5p
LINC01002	hsa-miR-7106-5p
XLOC_013274	hsa-miR-7106-5p
CYP4F35P	hsa-miR-7106-5p
RP11-15H20.6	hsa-miR-7106-5p

lncRNA	miRNA
C21orf15	hsa-miR-7106-5p
AC022007.5	hsa-miR-7106-5p
LINC00662	hsa-miR-7106-5p
LINC00662	hsa-miR-7106-5p
LINC00999	hsa-miR-7106-5p
CYP4F35P	hsa-miR-7106-5p
XLOC_006242	hsa-miR-7106-5p
AC138035.2	hsa-miR-7106-5p
LINC01001	hsa-miR-7106-5p
XLOC_008461	hsa-miR-7106-5p
RP11-15H20.6	hsa-miR-7106-5p
LINC00680	hsa-miR-7106-5p
FAM157C	hsa-miR-7106-5p
RP11-126K1.6	hsa-miR-7106-5p
CTBP1-AS2	hsa-miR-7106-5p
AP000251.3	hsa-miR-7106-5p
LINC01125	hsa-miR-7106-5p
AC009299.3	hsa-miR-7106-5p
AC009299.3	hsa-miR-7106-5p
XLOC_008528	hsa-miR-7106-5p
XLOC_001223	hsa-miR-7106-5p
TCL6	hsa-miR-7106-5p
CTB-89H12.4	hsa-miR-7106-5p
AC004951.6	hsa-miR-7106-5p
RP11-15H20.6	hsa-miR-7106-5p
LOC100506730	hsa-miR-7106-5p
ASB16-AS1	hsa-miR-7106-5p
AC022007.5	hsa-miR-7106-5p
RP11-384K6.6	hsa-miR-7106-5p
LINC00963	hsa-miR-7106-5p
LINC01002	hsa-miR-7106-5p
TTN-AS1	hsa-miR-7106-5p
RP11-155G14.6	hsa-miR-7106-5p
LINC01061	hsa-miR-7106-5p
XLOC_009783	hsa-miR-7106-5p
PPP3CB-AS1	hsa-miR-7106-5p
LINC01000	hsa-miR-7106-5p
PPP3CB-AS1	hsa-miR-7106-5p
RP11-617F23.1	hsa-miR-7106-5p
ERVK3-1	hsa-miR-7106-5p
RP11-15H20.6	hsa-miR-7106-5p

lncRNA	miRNA
AC003102.3	hsa-miR-7106-5p
CTC-365E16.1	hsa-miR-7106-5p
THUMPD3-AS1	hsa-miR-7106-5p
THUMPD3-AS1	hsa-miR-7106-5p
XLOC_006455	hsa-miR-7106-5p
SPACA6P	hsa-miR-7106-5p
RP11-22P6.3	hsa-miR-7106-5p
RP11-983P16.4	hsa-miR-7106-5p
XLOC_010268	hsa-miR-7106-5p
RP11-206L10.9	hsa-miR-7106-5p
GABPB1-AS1	hsa-miR-7106-5p
THUMPD3-AS1	hsa-miR-7106-5p
LINC00662	hsa-miR-7106-5p
AC138035.2	hsa-miR-7106-5p
LINC01128	hsa-miR-7106-5p
LAMTOR5-AS1	hsa-miR-7106-5p
LINC01001	hsa-miR-7106-5p
RP11-81H3.2	hsa-miR-7106-5p
RP11-504P24.8	hsa-miR-7106-5p
RP11-347C12.10	hsa-miR-7106-5p
LAMTOR5-AS1	hsa-miR-7106-5p
XLOC_003662	hsa-miR-7106-5p
ASB16-AS1	hsa-miR-7106-5p
LINC01001	hsa-miR-7106-5p
RP11-573D15.2	hsa-miR-7106-5p
LINC00494	hsa-miR-7106-5p
KCNK15-AS1	hsa-miR-7106-5p
LINC00265	hsa-miR-7106-5p
LINC00494	hsa-miR-7106-5p
LINC00173	hsa-miR-7106-5p
XIST	hsa-miR-7106-5p
XLOC_013998	hsa-miR-7106-5p
RP11-395P17.3	hsa-miR-7106-5p
RP11-15H20.6	hsa-miR-7106-5p
LINC00174	hsa-miR-7106-5p
LAMTOR5-AS1	hsa-miR-7106-5p
RP11-498C9.15	hsa-miR-7106-5p
RPARP-AS1	hsa-miR-7106-5p
BCYRN1	hsa-miR-7106-5p
LINC01002	hsa-miR-7106-5p
LINC00094	hsa-miR-7106-5p

lncRNA	miRNA
RP11-22P6.3	hsa-miR-7106-5p
LINC01002	hsa-miR-7106-5p
AC016747.3	hsa-miR-7106-5p
CTD-307407.12	hsa-miR-7106-5p
PCBP1-AS1	hsa-miR-7106-5p
LINC00094	hsa-miR-7106-5p
LINC01002	hsa-miR-7106-5p
LINC00174	hsa-miR-7106-5p
XLOC_009145	hsa-miR-7106-5p
LINC01002	hsa-miR-7106-5p
GLG1	hsa-miR-7106-5p
ZNF561-AS1	hsa-miR-7106-5p
RP11-15H20.6	hsa-miR-7106-5p
LINC01002	hsa-miR-7106-5p
LINC00960	hsa-miR-7106-5p
RP4-669L17.10	hsa-miR-7106-5p
LINC01002	hsa-miR-7106-5p
RP11-34P13.13	hsa-miR-7106-5p
FAM83H-AS1	hsa-miR-7106-5p
LINC00649	hsa-miR-7106-5p
CTC-459F4.3	hsa-miR-7106-5p
SLC25A25-AS1	hsa-miR-7106-5p
RP11-616M22.7	hsa-miR-7106-5p
LINC00963	hsa-miR-7106-5p
RP11-513I15.6	hsa-miR-7106-5p
RP11-6N17.4	hsa-miR-7106-5p
NUTM2B-AS1	hsa-miR-7106-5p
RP11-284F21.10	hsa-miR-7106-5p
RP11-21L23.2	hsa-miR-7106-5p
XLOC_002996	hsa-miR-7106-5p
LINC01002	hsa-miR-7106-5p
XLOC_014159	hsa-miR-7106-5p
GNAS-AS1	hsa-miR-7106-5p

Validation Of Lncrnas Expression Levels And Prognostic Roles

Subsequently, TANRIC database was used to detect the top twenty lncRNAs expression levels. As shown in Fig. S2A-S2I, Nine of twenty lncRNAs were significantly increased or downregulated between BC tissues and normal tissues. Furthermore, the prognostic functions of twenty lncRNAs of BC were also analyzed. As shown in Fig. S3A and S3B, the higher expression of both LINC00662 and LINC00963 significantly indicated a worse prognosis in BC.

Identification Of Upstream Transcription Factors Of De-mirnas

In current study, prediction of upstream TFs of screened DE-miRNAs was used by miRGen v3 database. Nine TFs for DE-miRNAs and corresponding motifs were presented in Fig. 2A-2I, which were NRF1, ELK4, E2F3, NR2F1, ZEB1, ZNF263, ZFX, POU2F2, and IRF1. As shown in Fig. 2J, NRF1 and ELK4 were the two most frequent predicted TFs of DE-miRNAs. The frequency of predicted TFs was also listed in Table 4.

Table 4
The prediction DE-miRNAs related transcription factors

TF name	Num of binding sites
NRF1	5
ELK4	4
E2F3	2
NR2F1	1
ZEB1	1
ZNF263	1
ZFX	1
POU2F2	1
IRF1	1

Identification Of Downstream Targeted Genes Of De-mirnas

It is well-known that miRNAs play their biological roles mainly by directly targeting 3' untranslated region of mRNA. Then, we verified the downstream targeted genes of candidate DE-miRNA through intersection of miRDB and miRTarBase on miRWalk database. 320 target genes were finally analyzed (Fig. 3A-3K) and listed in Table 5. Moreover, targeted gene counts for each DE-miRNA were simultaneously listed in Table 6.

Table 5
The prediction of DE-miRNAs related targeted genes

hsa-miR-423-5p	hsa-miR-3202	hsa-miR-419	hsa-miR-6750-5p	hsa-miR-4740-5p	hsa-miR-4779	hsa-miR-377-5p	hsa-miR-510-5p	hsa-miR-3613-3p	hsa-miR-6086	hsa-miR-7160-5p
STRN4	GPR107	GNS	FKBP4	SCD	KIF21B	SEC24A	HTR3E	MPRIP	TFPI	PAX2
GDF11	TOR1AIP2	ARL8B	PPIA	FKBP5	POLR2F	FKBP5	SLX4IP	CNOT6L	SAMD8	PLCD3
ASPH	SET	SESN3	QSER1	H6PD	IQSEC3	RC3H1	MRPS16	CCNY	ARHGEF2	CPSF2
HNRNPUL1	RNF187	C5orf51			SHISA6	TMEM30B	ZNF101	SLC24A4	RC3H1	AEHD12
NCS1	TBC1D2B	FKBP4			MTSS1L	GNL3L	ARIH1	SNIP1	IRGQ	VSIR
FOXK1	CAPZB				NOVA2	PDK3	TMED4	PSPC1	PLCXD1	USB1
MNT	FKBP4				TNRC6B	ZNF570	VHL	FKBP5	TMEM196	MSM01
ABCC5	SOCS7				RAB7A	CLEC7A	FKBP5	DYRK2	ZNF835	PDE4A
ARRB2	MLEC				URM1	RBMS2	SELENOH		TMEM30B	NECTIN1
KMT2B	TAOK1				SOX12	WDR26	ZNF410		CDADC1	TMEM63C
SEPT9	URM1				EPHB2		PRR14L		PDK3	PRX
HDGF	ZNF385A				SEMA4G		PPP2R5E		CARNMT1	SLC25A34
MFGE8	OTUD7B				CBX2		ZNF451		RNF126	TRIM65
ARHGDIA	FAM131B				HMGA1		UGGT1		ZNF587	PEX11B
TMEM184B	SYT7				CD3E				WDR26	PDE7A
SHANK3	PTPA				FKBP5				TMEM33	CYP51A1
C20orf27	SH3PXD2A				MIDN				CYTH2	PNMA2
PLCB1	SDK1				STMN1				CLEC7A	PDE7A
WFIKK2	SGK1								CNNM4	NOL9
RNF165	FOXJ2								NRXN3	CRIPT
SELENON	ATXN1L								ZNF385A	ZNF587
PDPK1	SEMA4G								FKBP5	ANGPT4
SRM	ABLIM1								RBMS2	TSKU
NAV1	ZBTB7B									TMEM209
CALM3	FSTL4									TRIM67
CCNF	UBE2V1									SNX27
MCRIP1	C6orf22									GLUL
LASP1	DNAJC8									ZNF329
MED28	ELFN2									RBMS2
SOX12	CNNM4									SENP5
MAP3K9	SLC26A9									SLC25A45
MKNK2	SZRD1									NFIC
STRIP2	TMEM167A									ITGA3
FKBP4	NAV2									PCYT1A
RNF187	TSEN54									RNF24
	CPM									PPFIBP1
	IP6K1									ATP1B4
	KDM5C									FZD7

hsa-miR-423-5p	hsa-miR-3202	hsa-miR-419	hsa-miR-6750-5p	hsa-miR-4740-5p	hsa-miR-4779	hsa-miR-377-5p	hsa-miR-510-5p	hsa-miR-3613-3p	hsa-miR-6086	hsa-miR-7160-5p
	ANKRD45									ZNF500
	PDXK									GLG1
	GPR173									SALL2
	FAM228A									SH3RF1
	TMEM9									TOR1AIP2
	MAFG									DTD2
	ZBTB33									CDCP1
	PLXNA2									CDKAL1
	NFAT5									NRIP3
	POTEG									ZNF490
										TOMM20
										HOOK3
										ANKRD40
										PAX5
										FBXO45
										SLC35E2
										CAPZB
										TRAF3IP2
										PTK6
										TMEM120B
										KLHDC10
										RPH3A
										BRBP
										TYRO3
										SLC38A7
										PHC2
										PHACTR4
										PHF12
										RAB33B
										PFKFB3
										PRELP
										CCND3
										NUP43
										TNN11
										FOXP4
										ZNF317
										TMEM239
										GTPBP1
										CCL22

hsa-miR-423-5p	hsa-miR-3202	hsa-miR-419	hsa-miR-6750-5p	hsa-miR-4740-5p	hsa-miR-4779	hsa-miR-377-5p	hsa-miR-510-5p	hsa-miR-3613-3p	hsa-miR-6086	hsa-miR-7160-5p
										HDAC5
										BTF3L4
										AMOTL1
										MINOS1-NBL1
										EFNA3
										ZNF674
										FNIP1
										PLAGL2
										ARRB1
										BVFS
										OPA3
										TGFBR3L
										SLC35F6
										KLC2
										ASB1
										RKP7A
										DNAL1
										GGCX
										SHISA6
										YWHAE
										TLCD2
										C11orf58
										PGAM5
										HECTK3
										NAV2
										SNX1
										ZCCHC24
										GOLGA3
										SLC19A3
										IQSEC3
										DNAJC24
										ATL3
										MOB3A
										IDS
										NPTXR
										NAV1
										GPRC5A
										RF41
										SAP18

hsa-miR-423-5p	hsa-miR-3202	hsa-miR-419	hsa-miR-6750-5p	hsa-miR-4740-5p	hsa-miR-4779	hsa-miR-377-5p	hsa-miR-510-5p	hsa-miR-3613-3p	hsa-miR-6086	hsa-miR-7160-5p
										UBXN2B
										CAMLG
										CLCC1
										RNF187
										LRKC59
										SLC11A2
										RGS6
										TVP23C
										SORCS2
										MYO1C
										NCEH1
										MAG
										ULK2
										DISC1
										SLC4A2
										CPM
										APOBFC3F
										BICDL1
										RAB21
										SUMF2
										S1PR2
										ATXN7L3
										UBE2D4
										DNAJC8
										ARMH3
										PACSIN1
										MSN
										NRF1
										ST7L
										NFAT5
										RBM43
										SLC2A4
										NTN1
										NBL1

Table 6
The predicted targeted gene count of each
DE-miRNA

miRNA ID	Target gene count
hsa-miR-423-5p	35
hsa-miR-3202	49
hsa-miR-4519	5
hsa-miR-6750-5p	3
hsa-miR-4740-5p	3
hsa-miR-4779	18
hsa-miR-377-5p	10
hsa-miR-510-5p	14
hsa-miR-3613-3p	8
hsa-miR-6086	23
hsa-miR-7106-5p	152

Screening Of Hub Genes

Furthermore, we mapped these candidate targeted genes based on the STRING database (Fig. S5A). The top ten hub genes were shown in Fig. S5B, which were RAB7A, CAPZB, SH3RF1, EPHB2, ARRB1, RNF126, ASB1, NTN1, POLR2F and SLC2A4.

Validation Of Hub Gene Expressions And Prognostic Roles

Using UALCAN database, we discovered that six of ten screened DE-miRNAs related hub genes were markedly upregulated in BC tissues than normal tissues. Three of ten screened DE-miRNAs related hub genes were significantly downregulated in BC tissues than normal tissues, whereas expression analysis of NTN1 showed no significant difference (Fig. 4A-4J).

To further identify potential hub genes, the prognostic functions of these hub genes in BC were conducted using bc-GenExMiner v4.2 database. As shown in Fig. 4K and 4L, a higher expression of RAB7A significantly indicated a worse prognosis while a higher expression of ARRB1 indicated a better prognosis of BC patients.

According to the predicted above-mentioned interactions, FKBP4 and FKBP5 related lncRNA-miRNA-mRNA regulatory axis related with development of BC were finally realized as presented in Fig. S6.

Discussion

It is widely acknowledged that there exist significant links between miRNA-mRNA regulatory axis and BC[16]. Recent studies have also suggested that lncRNAs could interact with other RNA transcripts via miRNA response element (MRE), which are proposed as the letters of a newfound RNA language[17]. For instance, lncRNA H19, transcriptional factor LIN28 as well as miRNA let-7 have been reported to form a double-negative regulatory network, which plays a pivotal role during the maintenance breast cancer stem cells[18]. FKBP5 have long been regarded as important regulators of the response to immunosuppressants FK506 and as molecular chaperones binding to different cellular receptors or targets[19]. More lately, various evidence has suggested that this complicated protein family might also play their roles in carcinogenesis, progression and chemoresistance of cancers[20–22].

Nevertheless, to our knowledge, a comprehensive FKBP-related ncRNA-mRNA regulatory axis in BC has not been established so far. In current study, we performed a differential expression analysis by using FKBP5 mRNA data of GEPIA database. Four FKBP4 related DE-miRNAs and seven FKBP5 related DE-miRNAs were eventually identified. Previous studies have demonstrated that most of expression and function of DE-miRNAs in tumors that we verified were identical with present analytic results. For instance, miR-423-5p is significantly upregulated among hepatocellular carcinoma (HCC) and enhance the proliferative and metastatic capacity of HCC cells[23]; tissue-specific and plasma miR-3613-3p has been found as a promising predictor in different staging lung squamous cell cancer[24].

Subsequently, by integrating DE-mRNAs and targeted lncRNAs of DE-miRNAs, expression and prognostic analytic results of nine in top twenty lncRNAs of BC were significantly identified. LINC00662 and LINC00963 expression were significantly associated with patients' OS, which were also identical with previous researches of various cancers. For instance, an investigation has lately demonstrated that high expression of LINC00662 contributed to

malignant proliferation of acute myeloid leukemia cells through upregulating ROCK1[25]. Moreover, LINC00963 was found to facilitate osteosarcoma growth and progression via inhibiting miR-204-3p/FN1 axis[26].

Previous researches have suggested that the expression of miRNA could be modulated by TFs[27]. Therefore, we predicted nine TFs potentially regulating above-mentioned DE-miRNAs. Nuclear factor erythroid 2-like 1 (NRF1, including a short form Nrf1 β /LCR-F1 and another long form TCF11)[28], was predicted as a TF potentially regulating expression of a relatively large proportion of screened DE-miRNAs. It has been demonstrated to act as an important player in regulating the expression and function of miRNAs. For example, a recent research has reported that NRF1 was participated in miR-219 signaling pathway, thereby inhibiting metastasis of BC cells[29]. Additionally, ETS-domain protein 4 (ELK4) was well elucidated to interact with miR-3188 in the development of atherosclerosis[30]. More researches on the functions of predicted TFs in BC are necessary to be further investigated.

Next, by integrating DE-mRNAs and targeted genes of DE-miRNAs, 320 candidate genes were identified. Subsequent GO and KEGG pathway analysis revealed that targeted genes were significantly enriched in cysteine-type endopeptidase activity involved in apoptotic process. A study performed by Siewiński et al. indicated that positive expression of high molecular weight cysteine proteinase inhibitor was observed on the tumor cell surface in serous and endometrioid metastatic ovarian cancer[31]. A plenty of investigations also suggested that apoptotic process correlated with BC[32–34], which further supported our current predicted findings.

Finally, PPI network was performed and top ten hub genes were verified. Moreover, differential expression analysis of these hub genes of BC were further conducted by using UALCAN database, including publicly available cancer OMICS data (TCGA and MET500). Inspiringly, most of these genes have been demonstrated to act as key regulators of BC. For instance, upregulated RAB7A was found correlated to poor prognosis of BC patients in this study, which is in accordance with the results of knockdown of RAB7A suppressing the proliferation and migration of BC cells[35]. In addition, analysis of hub genes’ prognostic functions also implied significant tumor suppressive effect of ARRB1 in BC, which is in accordance with research results of Son et al[36]. Based on above-mentioned findings, we established a predicted FKBP-related ncRNA-mRNA regulatory network, which could be very important for probing novel mechanisms and possible therapeutic targets of BC.

Conclusion

In current study, we firstly established a predicted FKBP-related ncRNA-mRNA regulatory network, thus exploring a comprehensive interpretation of molecular mechanisms and providing potential clues in seeking novel therapeutics for BC. In the future, much more experiments should be conducted to verify our findings.

Abbreviations

BC	breast cancer
FKBP	FK506-binding protein
DE-miRNA	differentially expressed miRNA
PPI	protein-protein Interaction
OS	overall survival
miRNA	microRNA
mRNA	messenger RNA
lncRNA	long non-coding RNA
TF	transcription factor
TANRIC	The Atlas of ncRNA in Cancer
BP	biological process
MF	molecular function
CC	cellular component
MRE	miRNA response element
HCC	hepatocellular carcinoma
NRF1	Nuclear factor erythroid 2-like 1
ELK4	ETS-domain protein 4

Declarations

Acknowledgments

We thank all authors for their critical reading and informative advice during the revision process. We apologize to all researchers whose relevant contributions were not cited due to space limitations.

Authors' contributions

Writing—Original Draft Preparation, X.H.C. and C.Z.H.; Writing—Review & Editing, Z.W.W., S.J., F.Q.S. and Y.X.F.; Funding Acquisition & Supervision, T.R.Y., C.J.D., X.S.D., W.L.B. and Z.J.C.. All authors have reviewed the manuscript.

Funding

The work was supported by the National Natural Science Foundation of China (No. 81972453, No. 81972597, No. 81672729 and No. 81602471), Zhejiang Provincial Natural Science Foundation of China under Grants (No. LY19H160055, No. LY19H160059, No. LY18H160030, No. LY18H160005, LY20H160026). The work was sponsored by Zheng Shu Medical Elite Scholarship Fund.

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have agreed to publish this manuscript.

Competing interests

The authors declare no conflict of interest.

References

1. Nagini S: **Breast Cancer: Current Molecular Therapeutic Targets and New Players**. *Anti-cancer agents in medicinal chemistry* 2017, **17**(2):152-163.
2. Sachs N, de Ligt J, Kopper O, Gogola E, Bounova G, Weeber F, Balgobind AV, Wind K, Gracanin A, Begthel H *et al*: **A Living Biobank of Breast Cancer Organoids Captures Disease Heterogeneity**. *Cell* 2018, **172**(1-2):373-386.e310.
3. Gharthey-Kwansah G, Li Z, Feng R, Wang L, Zhou X, Chen FZ, Xu MM, Jones O, Mu Y, Chen S *et al*: **Comparative analysis of FKBP family protein: evaluation, structure, and function in mammals and Drosophila melanogaster**. *BMC Dev Biol* 2018, **18**(1):7.
4. Ebong IO, Beilsten-Edmands V, Patel NA, Morgner N, Robinson CV: **The interchange of immunophilins leads to parallel pathways and different intermediates in the assembly of Hsp90 glucocorticoid receptor complexes**. *Cell discovery* 2016, **2**:16002.
5. Loh HY, Norman BP, Lai KS, Rahman N, Alitheen NBM, Osman MA: **The Regulatory Role of MicroRNAs in Breast Cancer**. *International journal of molecular sciences* 2019, **20**(19).
6. Xiong H, Zhao W, Wang J, Seifer BJ, Ye C, Chen Y, Jia Y, Chen C, Shen J, Wang L *et al*: **Oncogenic mechanisms of Lin28 in breast cancer: new functions and therapeutic opportunities**. *Oncotarget* 2017, **8**(15):25721-25735.
7. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z: **GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses**. *Nucleic acids research* 2017, **45**(W1):W98-w102.
8. Sticht C, De La Torre C, Parveen A, Gretz N: **miRWalk: An online resource for prediction of microRNA binding sites**. *PLoS One* 2018, **13**(10):e0206239.
9. Paraskevopoulou MD, Vlachos IS, Karagkouni D, Georgakilas G, Kanellos I, Vergoulis T, Zagganas K, Tsanakas P, Floros E, Dalamagas T *et al*: **DIANA-LncBase v2: indexing microRNA targets on non-coding transcripts**. *Nucleic acids research* 2016, **44**(D1):D231-238.
10. Georgakilas G, Vlachos IS, Zagganas K, Vergoulis T, Paraskevopoulou MD, Kanellos I, Tsanakas P, Dellis D, Fevgas A, Dalamagas T *et al*: **DIANA-miRGen v3.0: accurate characterization of microRNA promoters and their regulators**. *Nucleic acids research* 2016, **44**(D1):D190-195.
11. Li J, Han L, Roebuck P, Diao L, Liu L, Yuan Y, Weinstein JN, Liang H: **TANRIC: An Interactive Open Platform to Explore the Function of lncRNAs in Cancer**. *Cancer Res* 2015, **75**(18):3728-3737.
12. Huang da W, Sherman BT, Lempicki RA: **Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources**. *Nat Protoc* 2009, **4**(1):44-57.
13. Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, Santos A, Doncheva NT, Roth A, Bork P *et al*: **The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible**. *Nucleic acids research* 2017, **45**(D1):D362-d368.

14. Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi B, Varambally S: **UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses.** *Neoplasia (New York, NY)* 2017, **19**(8):649-658.
15. Jezequel P, Frenel JS, Campion L, Guerin-Charbonnel C, Gouraud W, Ricolleau G, Campone M: **bc-GenExMiner 3.0: new mining module computes breast cancer gene expression correlation analyses.** *Database : the journal of biological databases and curation* 2013, **2013**:bas060.
16. Zhu H, Dai M, Chen X, Chen X, Qin S, Dai S: **Integrated analysis of the potential roles of miRNAmRNA networks in triple negative breast cancer.** *Molecular medicine reports* 2017, **16**(2):1139-1146.
17. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP: **A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language?** *Cell* 2011, **146**(3):353-358.
18. Peng F, Li TT, Wang KL, Xiao GQ, Wang JH, Zhao HD, Kang ZJ, Fan WJ, Zhu LL, Li M *et al*: **H19/let-7/LIN28 reciprocal negative regulatory circuit promotes breast cancer stem cell maintenance.** *Cell death & disease* 2017, **8**(1):e2569.
19. McKeen HD, Brennan DJ, Hegarty S, Lanigan F, Jirstrom K, Byrne C, Yakkundi A, McCarthy HO, Gallagher WM, Robson T: **The emerging role of FK506-binding proteins as cancer biomarkers: a focus on FKBP.** *Biochemical Society transactions* 2011, **39**(2):663-668.
20. Tong J, Shen Y, Chen X, Wang R, Hu Y, Zhang X, Zhang Z, Han L: **FKBP3 mediates oxaliplatin resistance in colorectal cancer cells by regulating HDAC2 expression.** *Oncology reports* 2019.
21. Zhang Y, Zhang D, Lv J, Wang S, Zhang Q: **LncRNA SNHG15 acts as an oncogene in prostate cancer by regulating miR-338-3p/FKBP1A axis.** *Gene* 2019, **705**:44-50.
22. Zhu W, Li Z, Xiong L, Yu X, Chen X, Lin Q: **FKBP3 Promotes Proliferation of Non-Small Cell Lung Cancer Cells through Regulating Sp1/HDAC2/p27.** *Theranostics* 2017, **7**(12):3078-3089.
23. Wu LM, Ji JS, Yang Z, Xing CY, Pan TT, Xie HY, Zhang F, Zhuang L, Zhou L, Zheng SS: **Oncogenic role of microRNA-423-5p in hepatocellular carcinoma.** *Hepatobiliary & pancreatic diseases international : HBPD INT* 2015, **14**(6):613-618.
24. Pu Q, Huang Y, Lu Y, Peng Y, Zhang J, Feng G, Wang C, Liu L, Dai Y: **Tissue-specific and plasma microRNA profiles could be promising biomarkers of histological classification and TNM stage in non-small cell lung cancer.** *Thoracic cancer* 2016, **7**(3):348-354.
25. Liu Y, Gao X, Tian X: **High expression of long intergenic non-coding RNA LINC00662 contributes to malignant growth of acute myeloid leukemia cells by upregulating ROCK1 via sponging microRNA-340-5p.** *European journal of pharmacology* 2019, **859**:172535.
26. Zhou Y, Yin L, Li H, Liu LH, Xiao T: **The LncRNA LINC00963 facilitates osteosarcoma proliferation and invasion by suppressing miR-204-3p/FN1 axis.** *Cancer biology & therapy* 2019, **20**(8):1141-1148.
27. Si W, Shen J, Du C, Chen D, Gu X, Li C, Yao M, Pan J, Cheng J, Jiang D *et al*: **A miR-20a/ MAPK1/c-Myc regulatory feedback loop regulates breast carcinogenesis and chemoresistance.** *Cell death and differentiation* 2018, **25**(2):406-420.
28. Yuan J, Zhang S, Zhang Y: **Nrf1 is paved as a new strategic avenue to prevent and treat cancer, neurodegenerative and other diseases.** *Toxicology and applied pharmacology* 2018, **360**:273-283.
29. Xu Y, Luo Y, Liang C, Xing W, Zhang T: **A regulation loop between Nrf1alpha and MRTF-A controls migration and invasion in MDA-MB-231 breast cancer cells.** *International journal of molecular medicine* 2018, **42**(5):2459-2468.
30. Li N, Chen J, Zhao J, Wang T: **MicroRNA-3188 targets ETS-domain protein 4 and participates in RhoA/ROCK pathway to regulate the development of atherosclerosis.** *Die Pharmazie* 2017, **72**(11):687-693.
31. Siewinski M, Saleh Y, Popiela A, Ziolkowski P, Jelen M, Grybos M: **Expression of high molecular weight cysteine proteinase inhibitor in ovarian cancer tissues: regulation of cathepsin B expression by placental CPI.** *Biological chemistry* 2003, **384**(7):1103-1107.
32. Zhang YY, Shang XY, Hou XW, Li LZ, Wang W, Hayashi T, Zhang Y, Yao GD, Song SJ: **Yuanhuatane from Daphne genkwa selectively induces mitochondrial apoptosis in estrogen receptor alpha-positive breast cancer cells in vitro.** *Planta medica* 2019.
33. Raut GK, Chakrabarti M, Pamarthy D, Bhadra MP: **Glucose starvation induced upregulation of Prohibitin 1 via ROS generation causes mitochondrial dysfunction and apoptosis in breast cancer cells.** *Free radical biology & medicine* 2019.
34. Lee J, Park SH, Lee J, Chun H, Choi MK, Yoon JH, Pham TH, Kim KH, Kwon T, Ryu HW *et al*: **Differential effects of luteolin and its glycosides on invasion and apoptosis in MDA-MB-231 triple-negative breast cancer cells.** *EXCLI journal* 2019, **18**:750-763.
35. Xie J, Yan Y, Liu F, Kang H, Xu F, Xiao W, Wang H, Wang Y: **Knockdown of Rab7a suppresses the proliferation, migration, and xenograft tumor growth of breast cancer cells.** *Bioscience reports* 2019, **39**(2).
36. Son D, Kim Y, Lim S, Kang HG, Kim DH, Park JW, Cheong W, Kong HK, Han W, Park WY *et al*: **miR-374a-5p promotes tumor progression by targeting ARRB1 in triple negative breast cancer.** *Cancer letters* 2019, **454**:224-233.

Figures

Figure 1

Potential DE-miRNAs of FKBP4 and DE-miRNAs associated lncRNAs predicted by miRWalk and LncBase v.2 database. (A) DE-miRNAs-FKBP4 regulatory axis constructed by using miRWalk; (B) DE-miRNAs-FKBP5 regulatory axis constructed by using miRWalk; (C) Pie chart of top20 predicted lncRNAs interacted with DE-miRNAs.

Figure 2

Prediction of transcription factors of DE-miRNAs. (A-I) Nine transcription factors for DE-miRNAs and corresponding motifs; (J) Pie chart of frequency of transcription factors.

Figure 3

Predicted targeted genes of DE-miRNAs performed by miRWalk database. (A-K) Central blue dots are DE-miRNAs and surrounding yellow dots are potential target mRNAs.

Figure 4

Expression analysis and survival curves of hub genes of BC patients. (A-J) Expression analysis of ten hub genes of BC patient tissues; (K-L) Survival curves in BC patients are plotted significantly correlated with RAB7A and ARRB1.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supplementarymaterial.docx](#)