FKBP-related ncRNA-mRNA axis in breast cancer

CURRENT STATUS: UNDER REVIEW

Hanchu Xiong
Zhejiang University
ORCiD: 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 Corresponding Author
ORCiD: 0000-0002-0727-4034

DOI:
SUBJECT AREAS

Oncology  Cancer Biology

KEYWORDS

breast cancer; microRNA; long noncoding RNA; FK506-binding protein; bioinformatic analysis.
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 IncRNAs, 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 IncRNAs 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 IncRNAs were found for DE-miRNAs. Then, expression and prognostic results of nine of top twenty IncRNAs 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 FKBPs, 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 FKBP4s Expression Levels

The mRNA expressions of twelve FKBP4s 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 FKBP4 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 IncRNAs of screened miRNAs were all generated by using LncBase v.2, which is a tool used mainly for discovering the connection between miRNAs and IncRNAs[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 IncRNAs 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 IncRNAs of various cancer[11]. IncRNAs with |log2FC|>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 | 0.0295 | 0.991709556   | 30.3503   |
| hsa04971:Gastric acid secretion          | 4     | ATP1B4, CALM3, SLC4A2, PLCB1               | 0.0775 | 0.998436226   | 62.28624  |
| hsa04152:AMPK signaling pathway          | 5     | PDPK1, SLC2A4, PFKFB3, SCD, PPP2R5E         | 0.0872 | 0.992305724   | 66.77845  |
| hsa04960:Aldosterone-regulated sodium reabsorption | 3     | SGK1, PDPK1, ATP1B4                      | 0.0989 | 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 |log2FC|>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.

| miRNA   | RefseqID | GeneSymbol | Score | Position | Binding Site | Au     | Me      | N Pairings | TargetsMiRBase | TargetsMiRTarBase1 |
|---------|----------|-------------|-------|----------|--------------|--------|---------|------------|-----------------|------------------|
| hsa-miR-423-5p | NM_002014 | FKBP4 | 1      | 3'UTR    | 20,412,060 | 0.48   | -4.529  | 17         | LINK            | MIR038093         |
| hsa-miR-423-5p | NM_002014 | FKBP4 | 1      | 3'UTR    | 28,792,901 | 0.37   | -11.667 | 17         | LINK            | MIR038093         |
| hsa-miR-3202 | NM_002014 | FKBP4 | 1      | 3'UTR    | 29,572,977 | 0.46   | -7.861  | 15         | —               | MIR741143         |
| hsa-miR-3202 | NM_002014 | FKBP4 | 1      | 3'UTR    | 20,472,062 | 0.47   | -6.851  | 13         | —               | MIR741143         |
| hsa-miR-4519 | NM_002014 | FKBP4 | 1      | 3'UTR    | 24,172,451 | 0.4    | -6.2    | 14         | —               | MIR745785         |
| hsa-miR-6750-5p | NM_002014 | FKBP4 | 1      | 3'UTR    | 21,022,125 | 0.46   | -10.109 | 20         | —               | MIR744571         |
Table 2
The predicted targeted DE-miRNAs of FKBP5

| miRNA     | RefseqID   | GeneSymbol | Score | Position | Binding Site | Au    | Me    | N Pairings | Targets | 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_0041117   | FKBP5      | 1     | 3UTR     | 16,961,711   | 0.34  | -16.423| 14         | --      | Link  | MIRT537338 |
| hsa-miR-4779   | NM_0041117   | 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 IncRNAs were also listed in Table 3.

Table 3
| lncRNA          | miRNA       |
|----------------|-------------|
| UCA1           | hsa-miR-423-5p |
| KCNQ1OT1       | 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 |
| KCNQ1OT1       | 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  |
| 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  |
| RP11-545S15.3  | 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|
| RP11-93209.9  | 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|
| 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-6750-5p|
| UCA1          | hsa-miR-6750-5p|
| CTD-2017D11.1 | hsa-miR-6750-5p|
| LOC100506639  | hsa-miR-6750-5p|
| RP11-658F2.8  | hsa-miR-6750-5p|
| RP11-440L14.1 | hsa-miR-6750-5p|
| A1BG-AS1      | hsa-miR-6750-5p|
| A1BG-AS1      | hsa-miR-6750-5p|
| CTD-2017D11.1 | hsa-miR-6750-5p|
| ARHGEF26-AS1  | hsa-miR-6750-5p|
| XIST          | hsa-miR-6750-5p|
| RP4-806M20.3  | hsa-miR-6750-5p|
| XIST          | hsa-miR-6750-5p|
| KCNQ1OT1      | hsa-miR-6750-5p|
| RPS-890E16.2  | hsa-miR-6750-5p|
| TMPO-AS1      | hsa-miR-6750-5p|
| TMPO-AS1      | hsa-miR-6750-5p|
| XLOC_006476   | hsa-miR-4779 |
| RP11-10L12.4  | hsa-miR-4779 |
| KCNQ1OT1      | 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 |
| 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 |
| 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 |
| FGDS5-AS1 | hsa-miR-3613-3p |
| RP11-159D12.2 | hsa-miR-3613-3p |
| LINC01087 | hsa-miR-3613-3p |
| LRRCC75A-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 |
| ACC025335.1 | hsa-miR-3613-3p |
| LINC00963 | hsa-miR-3613-3p |
| BCYRN1 | hsa-miR-3613-3p |
| LRRCC75A-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 |
| LINCC0342 | hsa-miR-3613-3p |
| CTD-3092A11.2 | hsa-miR-3613-3p |
| LINCC0038 | 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 |
| LRRCC75A-AS1 | hsa-miR-3613-3p |
| CTD-2369P2.2 | hsa-miR-3613-3p |
| LRRCC75A-AS1 | hsa-miR-3613-3p |
| FLJ31306 | hsa-miR-3613-3p |
| KB-1460A1.5 | hsa-miR-3613-3p |
| LINCC00680 | 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 |
| LINCC00938 | 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 |
| LRRCC75A-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 |
| Gene Name       | miRNA     |
|----------------|----------|
| 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 |
| FLI10038       | hsa-miR-3613-3p |
| LINC00467      | hsa-miR-3613-3p |
| RP11-159D12.2  | hsa-miR-3613-3p |
| XLOC 010212    | hsa-miR-3613-3p |
| LRRCC75A-AS1   | hsa-miR-3613-3p |
| LRRCC75A-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-A51     | hsa-miR-3613-3p |
| FAM211A-AS1    | hsa-miR-3613-3p |
| XLOC_008461    | hsa-miR-3613-3p |
| LRRCC75A-AS1   | hsa-miR-3613-3p |
| RP11-395P17.3  | hsa-miR-3613-3p |
| LINC00963      | hsa-miR-3613-3p |
| LRRCC75A-AS1   | hsa-miR-3613-3p |
| LRRCC75A-AS1   | hsa-miR-3613-3p |
| MIR17HG        | hsa-miR-3613-3p |
| LINC00630      | hsa-miR-3613-3p |
| PCAT7          | hsa-miR-3613-3p |
| LRRCC75A-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-A51   | hsa-miR-3613-3p |
| SNHG16         | hsa-miR-3613-3p |
| AC013994.2     | 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 |
| LRRCC75A-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 |
| 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 |
| LINC0052       | hsa-miR-3613-3p |
| TMEM191C       | hsa-miR-3613-3p |
| RP11-443B7.1   | hsa-miR-3613-3p |
| LRRCC75A-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 |

14
| Gene          | miRNA     |
|--------------|-----------|
| 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 |
| RP11-222P7.2 | hsa-miR-6086 |
| TTN-AS1      | hsa-miR-6086 |
| RP11-34P13.13| hsa-miR-7106-5p |
| LOC100190986 | hsa-miR-7106-5p |
| KCNQ1OT1     | 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 |
| 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 |
| CTBPI-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_00852B  | 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 |
| 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 |
| Gene Symbol | miRNA Target |
|-------------|--------------|
| 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 |
| RP11-22P6.3 | hsa-miR-7106-5p |
| LINC01002   | hsa-miR-7106-5p |
| LINC00094   | 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 |
| LINC00963   | 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 IncRNAs expression levels. As shown in Fig. S2A-S2I, Nine of twenty IncRNAs were significantly increased or downregulated between BC tissues and normal tissues. Furthermore, the prognostic functions of twenty IncRNAs 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.

| 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.

| TF name | Num of binding sites |
|---------|----------------------|
| NRF1    |                      |
| ELK4    |                      |
| E2F3    |                      |
| NR2F1   |                      |
| ZEB1    |                      |
| ZNF263  |                      |
| ZFX     |                      |
| POU2F2  |                      |
| IRF1    |                      |
| hsa-miR-423-5p | hsa-miR-3202 | 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        |
| GDF11         | TORS1AIP2   | ARL8B          | PPIA           | FKBp5       | PLOLR2F     | SLC4IP      | CNOT6L      | SAMD8       | PAX2        |
| ASPH          | SET         | SESN3          | QSER1          | H6PD        | IQSEC3      | RC3H1       | MRPS16      | CCNY        | ARHGEF5     |
| HNRNPUL1      | RNF187      | C5orf51        | STRN4          | STRN4       | STRN4       | STRN4       | STRN4       | STRN4       | STRN4       |
| NCS1          | TBC1D2      | FKBp4          | MTSS1L         | GNL3L       | ARH1        | SNIP1       | IRGQ        | VSIR        | VSIR        |
| FOXK1         | CAPZB       | NOVA2          | PDK3           | TMED4       | PSCP1       | PLCXD1      | USB1        | MSMO1       | MSMO1       |
| MNT           | FKBp4       | TNRC6B         | ZNF570         | VHL         | FKBp5       | DYSK2       | ZNF835      | PDE4A       | PDE4A       |
| ABC50         | SOCS7       | RAB7A          | CLEC7A         | FKBp5       | DYSK2       | ZNF835      | PDE4A       | PDE4A       | PDE4A       |
| ARBB2         | MLEC        | URM1           | RBMS2          | SELENOH     | NECTIN1     | TMEM30B     | NECTIN1     | NECTIN1     | NECTIN1     |
| KMT2B         | TAOK1       | SOX12          | WDR26          | ZNF410      | CDADC1      | TMEM63C     | TMEM63C     | TMEM63C     | TMEM63C     |
| SEPT9         | URM1        | EPHB2          | PRRI14L        | PDK3        | PRX         | CARYMT1     | SLC25A34    | SLC25A34    | SLC25A34    |
| HDGF          | ZNF385A     | SEMA4G         | PPP2R5E        | CARNMT1     | PDE4A       | SLC25A34    | SLC25A34    | SLC25A34    | SLC25A34    |
| MEGF8         | OTUD7B      | CBX2           | ZNF451         | RNF126      | TRIM65      | RNF126      | TRIM65      | TRIM65      | TRIM65      |
| ARHGD1        | FAM131      | CBX2           | ZNF451         | RNF126      | TRIM65      | RNF126      | TRIM65      | TRIM65      | TRIM65      |
| TMEM184B      | SYT7        | CD3E           | WDR26          | PDE7A       | PDE7A       | PDE7A       | PDE7A       | PDE7A       | PDE7A       |
| SHANK3        | PTPA        | FKBp5          | TMEM33         | CYP51A1     | PDE7A       | PDE7A       | PDE7A       | PDE7A       | PDE7A       |
| C2orf27       | SH3PXD2A    | MIDN           | TMEM33         | CYP51A1     | PDE7A       | PDE7A       | PDE7A       | PDE7A       | PDE7A       |
| PLCB1         | SDK1        | STMN1          | CLEC7A         | PDE7A       | PDE7A       | PDE7A       | PDE7A       | PDE7A       | PDE7A       |
| WIFKKN2       | SGK1        | STMN1          | CNNM4          | NOL9        | CNNM4       | NOL9        | CNNM4       | NOL9        | NOL9        |
| RNF165        | FOXJ2       | ATXN1L         | NRXN3          | CRIP1       | NRXN3       | CRIP1       | NRXN3       | CRIP1       | CRIP1       |
| SELENO        | ATXN1L      | ATXN1L         | ZNF385A        | ZNF587      | ZNF587      | ZNF587      | ZNF587      | ZNF587      | ZNF587      |
| PDPK1         | SEMA4G      | FKBp5          | TMEM33         | CYP51A1     | PDE7A       | PDE7A       | PDE7A       | PDE7A       | PDE7A       |
| SRM           | ABLIM1      | RBMS2          | TMEM33         | CYP51A1     | PDE7A       | PDE7A       | PDE7A       | PDE7A       | PDE7A       |
| NAV1          | ZBTB7B      | TMEM209        | TMEM209        | TMEM209     | TMEM209     | TMEM209     | TMEM209     | TMEM209     | TMEM209     |
| CALM3         | FSTL4       | TRIM67         | SNX27          | GLUL        | ZNF329      | ZNF329      | ZNF329      | ZNF329      | ZNF329      |
| CCNF          | UBE2V1      | TRIM67         | SNX27          | GLUL        | ZNF329      | ZNF329      | ZNF329      | ZNF329      | ZNF329      |
| MCRIP1        | C6orf22     | TRIM67         | SNX27          | GLUL        | ZNF329      | ZNF329      | ZNF329      | ZNF329      | ZNF329      |
| LASP1         | DNAIC8      | TRIM67         | SNX27          | GLUL        | ZNF329      | ZNF329      | ZNF329      | ZNF329      | ZNF329      |
| MED28         | ELF2N2      | RBMS2          | SENP5          | SLC25A45    | SLC25A45    | SLC25A45    | SLC25A45    | SLC25A45    | SLC25A45    |
| SOX12         | CNNM4       | RBMS2          | SENP5          | SLC25A45    | SLC25A45    | SLC25A45    | SLC25A45    | SLC25A45    | SLC25A45    |
| MAPK9         | SLC26A9     | ZNF500         | ZNF500         | ZNF500      | ZNF500      | ZNF500      | ZNF500      | ZNF500      | ZNF500      |
| MKNK2         | SGRD1       | NFIC           | NFIC           | NFIC        | NFIC        | NFIC        | NFIC        | NFIC        | NFIC        |
| STRIP2        | TMEM167A    | ITGA3          | ITGA3          | ITGA3       | ITGA3       | ITGA3       | ITGA3       | ITGA3       | ITGA3       |
| FKBp4         | NAV2        | PCYT1A         | PCYT1A         | PCYT1A      | PCYT1A      | PCYT1A      | PCYT1A      | PCYT1A      | PCYT1A      |
| RNF187        | TSEN54      | RNF24          | RNF24          | RNF24       | RNF24       | RNF24       | RNF24       | RNF24       | RNF24       |
| CPM           | IP6K1       | ATP1B1P        | ATP1B1P        | ATP1B1P     | ATP1B1P     | ATP1B1P     | ATP1B1P     | ATP1B1P     | ATP1B1P     |
| KDM5C         | ANKRD45     | FZD7           | FZD7           | FZD7        | FZD7        | FZD7        | FZD7        | FZD7        | FZD7        |
| PDAXK         | GPR173      | ZNF500         | ZNF500         | ZNF500      | ZNF500      | ZNF500      | ZNF500      | ZNF500      | ZNF500      |
| GPR173        | FAM228A     | GLC1           | GLC1           | GLC1        | GLC1        | GLC1        | GLC1        | GLC1        | GLC1        |
| MAFG          | ZBTB33      | SALL2          | SALL2          | SALL2       | SALL2       | SALL2       | SALL2       | SALL2       | SALL2       |
| ZBTB33        | PLXNA2      | SH3RF1         | SH3RF1         | SH3RF1      | SH3RF1      | SH3RF1      | SH3RF1      | SH3RF1      | SH3RF1      |
| NFAT5         | POTEG       | ZNF490         | ZNF490         | ZNF490      | ZNF490      | ZNF490      | ZNF490      | ZNF490      | ZNF490      |
| Gene   |
|--------|
| 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  |
| HDAC5  |
| BTF3L4 |
| AMOTL1 |
| MINOS1-NBL1|
| EFNA3 |
| ZNF674 |
| FNIP1  |
| PLAGL2 |
| ARRB1  |
| BVFS   |
| OPA3   |
| TGFBR3L|
| SLC35F6|
| KLC2   |
| ASB1   |
| RKP7A  |
| DNAL1  |
| GCCX   |
| SHISA6 |
| YWHAE  |
| TLCD2  |
| C11orf58|
| PGAM5  |
| HECTK3 |
| NAV2   |
| SNX1   |
| ZCCHC2 |
| Owners |
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, PO LR2F 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 IncRNA-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 IncRNAs 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, IncRNA H19, transcriptional factor LIN28 as well as miRNA let-7 have been reported to form a double-negative regulatory network, which palys a pivotal role during the maintenance breast cancer stem cells[18]. FKBPs 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 FKBPs mRNA data of GEPIA database. Four FKBPs related DE-miRNAs and seven FKBPs 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 Nrf1β/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 IncRNAs 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 miRNA-mRNA 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 FKBPL. 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
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: Yuanhuatine 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 FKBPs and DE-miRNAs associated IncRNAs 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 IncRNAs 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 the primary manuscript. Click to download.

supplementary material.docx