

FKBP-related ncRNA-mRNA axis in breast cancer

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Abstract

Background: Breast cancer (BC) is a disease with morbidity ranking the first of women world widely. 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.

Keywords: Breast cancer; MicroRNA; Long noncoding RNA; FK506-binding protein; Bioinformatic analysis.

Introduction

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 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

DAVID database (<https://david.ncifcrf.gov/home.jsp>) was used to perform GO annotation and KEGG pathway analysis for the targeted genes [12]. The GO analysis consists of three categories: biological process (BP), molecular function (MF), as well as cellular component (CC).

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].

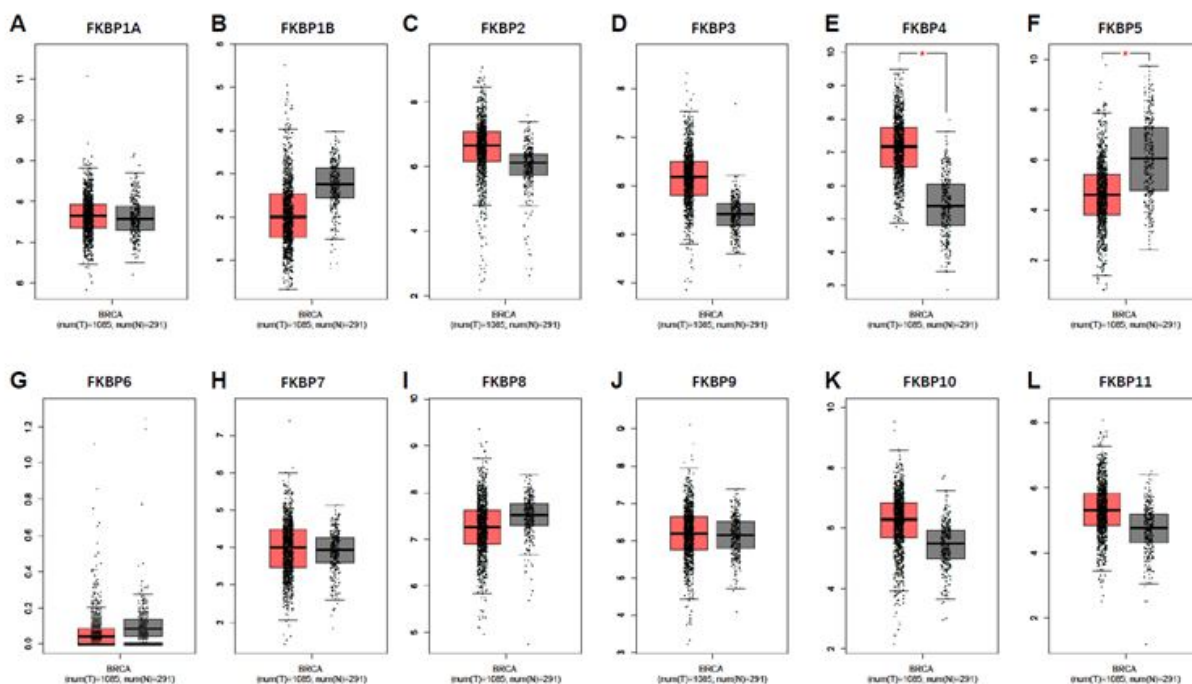
Statistical Analysis

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 Table 1 and 2.



FigureS1: Differential expressions of twelve FKBP family genes on the GEPIA database. (A-D, G-L) The expression levels of 10 FKBP's have no significant change between tumor group (T) and normal group (N); (E, F) The expression levels of FKBP4 and FKBP5 have significant changes between tumor group (T) and normal group (N), *P<0.05.

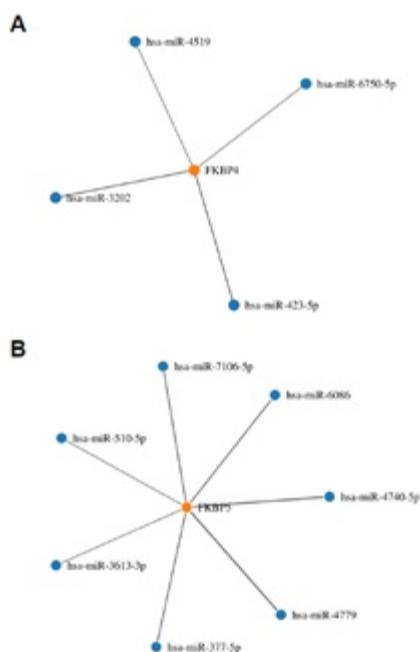


Figure1: Potential DE-miRNAs of FKBP's and DE-miRNAs associated lncRNAs predicted by miRWalk and LncBase v.2 database. (A) DE-miRNAs-FKBP4 regulatory axis constructed by using miRWalk; (B) DE-miRNAs-FKBP5 regulatory axis constructed by using miRWalk

Table 1: The predicted targeted DE-miRNAs of FKBP4

| miRNA | RefseqID | Gene Symbol | 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.

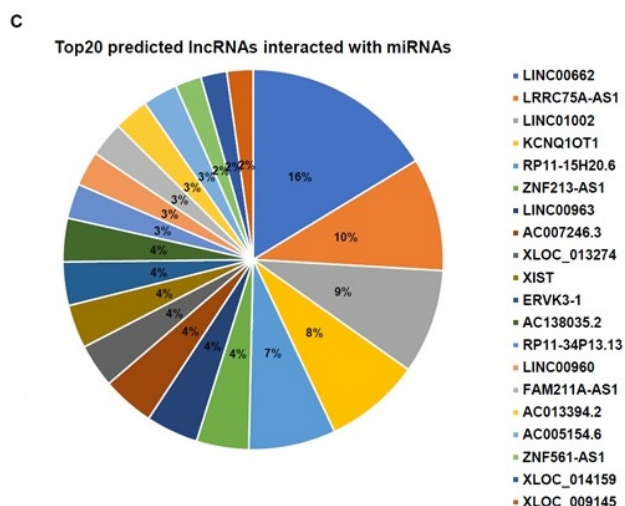


Figure 1: Potential DE-miRNAs of FKBP3 and DE-miRNAs associated lncRNAs predicted by miRWalk and LncBase v.2 database. (C) Pie chart of top20 predicted lncRNAs interacted with DE-miRNAs.

Table 3: The predicted targeted DE-miRNAs associated lncRNAs

| 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 |
| RP11-545I5.3 | 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 |
| 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 |

| | |
|---------------|-----------------|
| KCNQ1OT1 | 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 |
| 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 |
| XLOC_014159 | hsa-miR-4779 |
| LINC00680 | hsa-miR-4779 |
| LINC00662 | hsa-miR-377-5p |
| LINC00662 | hsa-miR-377-5p |
| LINC00662 | hsa-miR-377-5p |
| KCNQ1OT1 | 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 |
| KCNQ1OT1 | hsa-miR-510-5p |
| 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 |

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|---------------|-----------------|
| 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 |
| KCNQ1OT1 | 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 |
| 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 |
| LRRC75A-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 |

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|---------------|-----------------|
| 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 |
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| LRRC75A-AS1 | hsa-miR-3613-3p |
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| 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 |
| 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 |
| LRRC75A-AS1 | hsa-miR-3613-3p |
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| LRRC75A-AS1 | hsa-miR-3613-3p |
| FLJ31306 | hsa-miR-3613-3p |
| KB-1460A1.5 | hsa-miR-3613-3p |
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| ZNF213-AS1 | hsa-miR-3613-3p |
| LINC01355 | hsa-miR-3613-3p |
| LINC01002 | hsa-miR-3613-3p |
| GLG1 | hsa-miR-3613-3p |
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| RP11-46C24.7 | hsa-miR-3613-3p |

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| AC083843.1 | hsa-miR-3613-3p |
| NEAT1 | hsa-miR-3613-3p |
| LRRC75A-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 |
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| XLOC_007970 | hsa-miR-3613-3p |
| RP11-6N17.4 | hsa-miR-3613-3p |
| NUTM2B-AS1 | hsa-miR-3613-3p |
| 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 |

| | |
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| 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 |
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| 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 |
| 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 |
| KCNQ1OT1 | hsa-miR-6086 |
| RP11-15H20.6 | hsa-miR-6086 |

| | |
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| 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 |
| KCNQ1OT1 | hsa-miR-7106-5p |
| ZNF561-AS1 | hsa-miR-7106-5p |
| LINC00174 | hsa-miR-7106-5p |
| AC138035.2 | hsa-miR-7106-5p |
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| 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 |
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| CYP4F35P | hsa-miR-7106-5p |
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| AC138035.2 | hsa-miR-7106-5p |
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| 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 |
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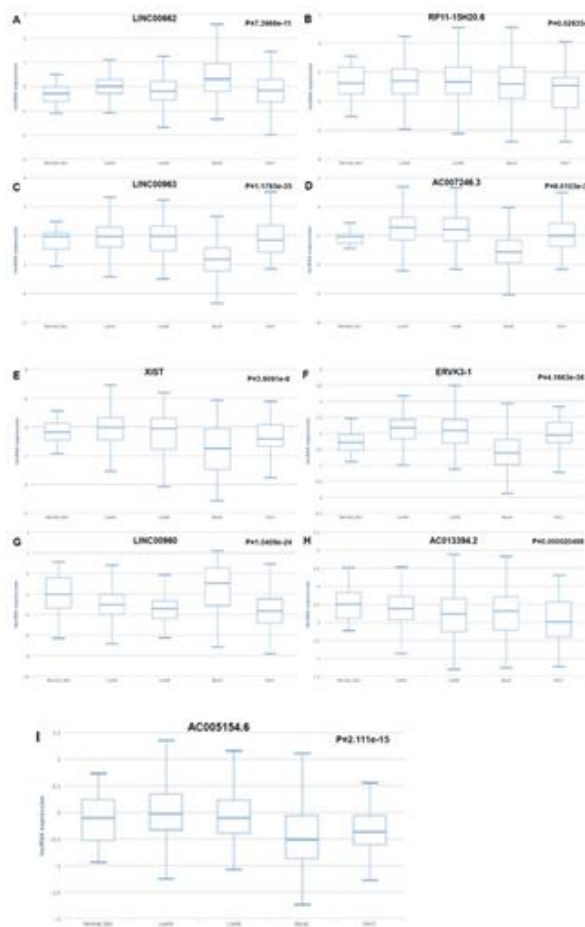
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| TCL6 | hsa-miR-7106-5p |
| CTB-89H12.4 | hsa-miR-7106-5p |
| AC004951.6 | hsa-miR-7106-5p |
| RP11-15H20.6 | hsa-miR-7106-5p |
| LOC100506730 | hsa-miR-7106-5p |
| ASB16-AS1 | hsa-miR-7106-5p |
| AC022007.5 | hsa-miR-7106-5p |
| RP11-384K6.6 | hsa-miR-7106-5p |
| 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 |
| 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 |
| RP11-22P6.3 | hsa-miR-7106-5p |
| LINC01002 | hsa-miR-7106-5p |
| AC016747.3 | hsa-miR-7106-5p |
| CTD-3074O7.12 | 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 |

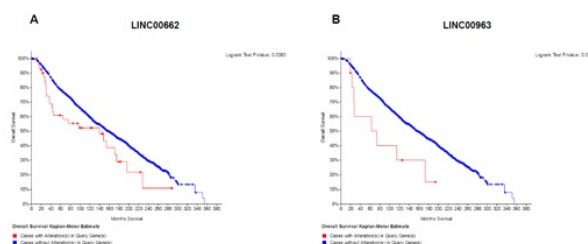
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| RP4-669L17.10 | hsa-miR-7106-5p |
| LINC01002 | hsa-miR-7106-5p |
| RP11-34P13.13 | hsa-miR-7106-5p |
| FAM83H-AS1 | hsa-miR-7106-5p |
| LINC00649 | hsa-miR-7106-5p |
| CTC-459F4.3 | hsa-miR-7106-5p |
| SLC25A25-AS1 | hsa-miR-7106-5p |
| RP11-616M22.7 | 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.



FigureS2: lncRNAs expression analysis in four subtypes of BC (TANRIC database). (A-F) Six significantly upregulated lncRNAs in BC tissues; (G-I) Three significantly downregulated lncRNAs in BC tissues.



FigureS3: Survival curves in BC patients are plotted correlated with (A) LINC00662 and (B) LINC00963.

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**.

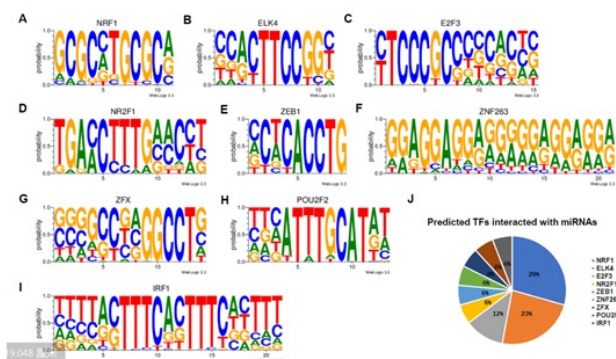


Figure2: Prediction of transcription factors of DE-miRNAs. (A-I) Nine transcription factors for DE-miRNAs and corresponding motifs; (J) Pie chart of frequency of transcription factors.

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.

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 |

Then, we verified the downstream targeted genes of candidate DE-miRNA through intersection of miRDB and miRTarBase on miWalk 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**.

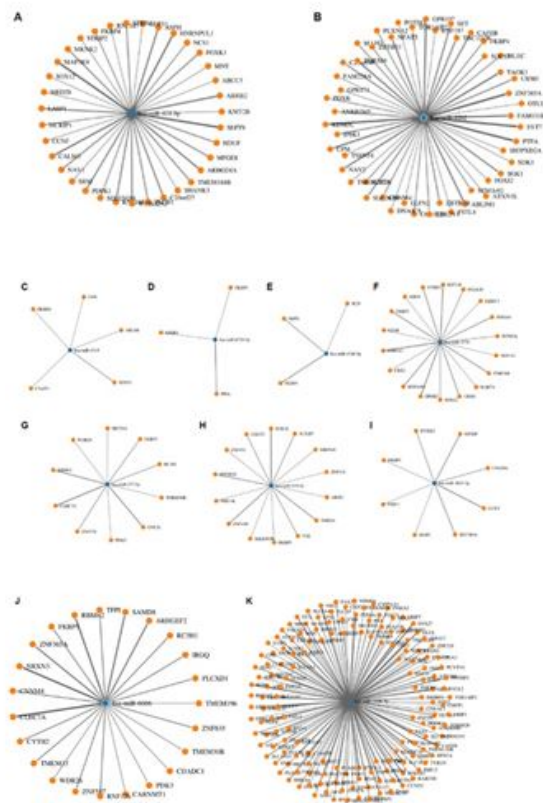
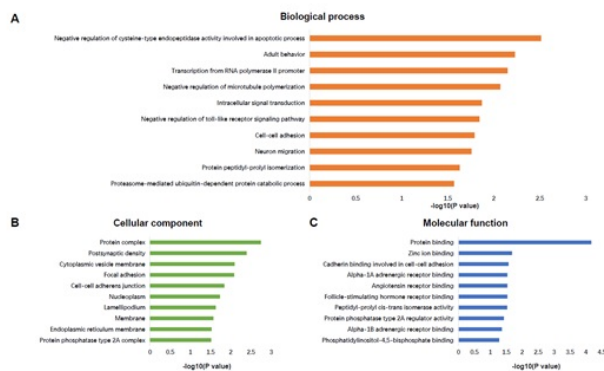


Figure3: Predicted targeted genes of DE-miRNAs performed by miWalk database. (A-K) Central blue dots are DE-miRNAs and surrounding yellow dots are potential target mRNAs.

| | | | | | | | | | | |
|----------|----------|--|--|--|--------|--------|---------|-------|---------|----------|
| MNT | FKBP4 | | | | TNRC6B | ZNF570 | VHL | FKBP5 | TMEM196 | MSMO1 |
| ABCC5 | SOCS7 | | | | RAB7A | CLEC7A | FKBP5 | DYRK2 | ZNF835 | PDE4A |
| ARRB2 | MLEC | | | | URM1 | RBMS2 | SELENOH | | TMEM30B | NECTIN1 |
| KMT2B | TAOK1 | | | | SOX12 | WDR26 | ZNF410 | | CDADC1 | TMEM63C |
| 9-Sep | URM1 | | | | EPHB2 | | PRR14L | | PDK3 | PRX |
| HDGF | ZNF385A | | | | SEMA4G | | PPP2R5E | | CARNMT1 | SLC25A34 |
| MFGE8 | OTUD7B | | | | CBX2 | | ZNF451 | | RNF126 | TRIM65 |
| ARHGDI3 | 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 |
| WFIKKN2 | SGK1 | | | | | | | | CNNM4 | NOL9 |
| RNF165 | FOXJ2 | | | | | | | | NRXN3 | CRIP1 |
| 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 |
| | ANKRD45 | | | | | | | | | ZNF500 |
| | PDXK | | | | | | | | | GLG1 |
| | GPR173 | | | | | | | | | SALL2 |
| | FAM228A | | | | | | | | | SH3RF1 |
| | TMEM9 | | | | | | | | | TOR1AIP2 |
| | MAFG | | | | | | | | | DTD2 |
| | ZBTB33 | | | | | | | | | CDCP1 |

| | | | | | | | | | | |
|--|--|--|--|--|--|--|--|--|--|----------|
| | | | | | | | | | | 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 |
| | | | | | | | | | | UBXN2B |
| | | | | | | | | | | CAMLG |
| | | | | | | | | | | CLCC1 |
| | | | | | | | | | | RNF187 |
| | | | | | | | | | | LRKC59 |
| | | | | | | | | | | SLC11A2 |
| | | | | | | | | | | RGS6 |

As shown in **Table 7**, candidate targeted genes of screened DE-miRNAs were markedly enriched in Axon guidance.



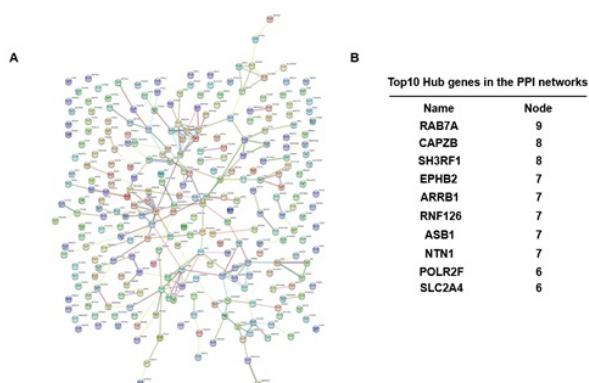
FigureS4: GO analysis of candidate genes. (A) The top ten enriched BP items of predicted genes; (B) The top ten enriched CC items of predicted genes; (C) The top ten enriched MF items of predicted genes.

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 | 0.9917096 | 30.3503 |
| hsa04971:Gastric acid secretion | 4 | ATP1B4, CALM3, SLC4A2, PLCB1 | 0.077583 | 0.9984362 | 62.28624 |
| hsa04152:AMPK signaling pathway | 5 | PDPK1, SLC2A4, PFKFB3, SCD, PPP2R5E | 0.087221 | 0.9923057 | 66.77845 |
| hsa04960:Aldosterone-regulated sodium reabsorption | 3 | SGK1, PDPK1, ATP1B4 | 0.098903 | 0.9844808 | 71.56362 |

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.



FigureS5: Protein to protein Interactions among candidate genes and screening of hub genes. (A) PPI networks of targeted genes of DE-miRNAs; (B) Screened hub genes of the PPI networks.

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**.

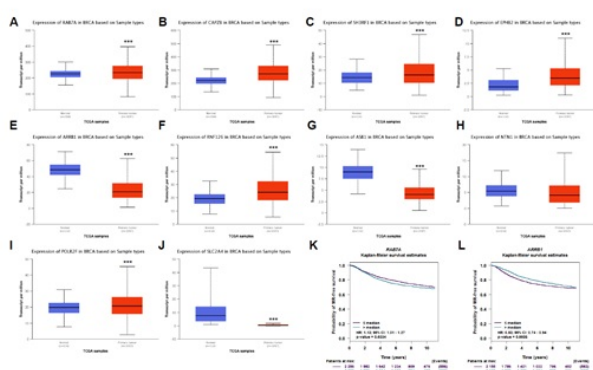
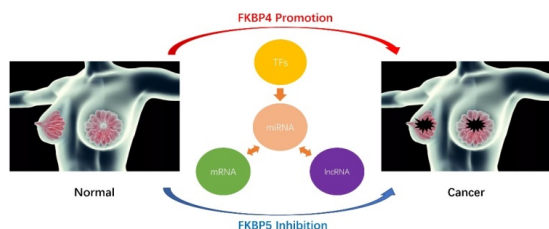


Figure4: Expression analysis and survival curves of hub genes of BC patients. (A-J) Expression analysis of ten hub genes of BC patient tissues; (K-L) Survival curves in BC patients are plotted significantly correlated with RAB7A and ARRB1.



FigureS6: The predicted TFs-miRNA-lncRNA/mRNA regulatory network of FKBP4 and FKBP5 in BC. FKBP4 promotes breast cancer and FKBP5 inhibits breast cancer via multiple key TFs-miRNA-lncRNA/mRNA interactions.

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.

Author Contributions

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

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Conflicts of Interest

The authors declare no conflict of interest.

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