Clinical Value of miR-101-3p and Biological Analysis of its Prospective Targets in Breast Cancer: A Study Based on The Cancer Genome Atlas (TCGA) and Bioinformatics

Background: MiR-101-3p can promote apoptosis and inhibit proliferation, invasion, and metastasis in breast cancer (BC) cells. However, its mechanisms in BC are not fully understood. Therefore, a comprehensive analysis of the target genes, pathways, and networks of miR-101-3p in BC is necessary.

Material/Methods: The miR-101 profiles for 781 patients with BC from The Cancer Genome Atlas (TCGA) were analyzed. Gene expression profiling of GSE31397 with miR-101-3p transfected MCF-7 cells and scramble control cells was downloaded from Gene Expression Omnibus (GEO), and the differentially expressed genes (DEGs) were identified. The potential genes targeted by miR-101-3p were also predicted. Gene Ontology (GO) and pathway and network analyses were constructed for the DEGs and predicted genes.

Results: In the TCGA data, a low level of miR-101-2 expression might represent a diagnostic (AUC: 0.63) marker, and the miR-101-1 was a prognostic (HR=1.79) marker. MiR-101-1 was linked to the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), and miR-101-2 was associated with the tumor (T), lymph node (N), and metastasis (M) stages of BC. Moreover, 427 genes were selected from the 921 DEGs in GEO and the 7924 potential target genes from the prediction databases. These genes were related to transcription, metabolism, biosynthesis, and proliferation. The results were also significantly enriched in the VEGF, mTOR, focal adhesion, Wnt, and chemokine signaling pathways.

Conclusions: MiR-101-1 and miR-101-2 may be prospective biomarkers for the prognosis and diagnosis of BC, respectively, and are associated with diverse clinical parameters. The target genes of miR-101-3p regulate the development and progression of BC. These results provide insight into the pathogenic mechanism and potential therapies for BC.

MeSH Keywords: Breast Neoplasms • Gene Expression Profiling • Gene Targeting • Information Systems • MicroRNAs

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* These authors contributed equally to this work

Corresponding Authors: Hua-wei Zhu, e-mail: 526202641@qq.com, Gang Chen, e-mail: chen_gang_triones@163.com

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Background

Breast cancer (BC), the most frequent carcinoma diagnosed and cause of death among women, affects 1,676,660 women worldwide and causes 521,900 mortalities every year [1]. The latest statistics have revealed that the morbidity and mortality rates associated with BC accounts for 29% and 14% of cancers, respectively, in the United States [2]. Similarly, the incidence of BC in China is also increasing annually [3]. Targeted drugs have been approved to treat HER2-positive BC, but resistance remains an inevitable outcome [4]. BC is a molecularly heterogeneous disease [5], and well-defined and effective molecular targets are still lacking. Therefore, the need to identify novel therapeutic targets for BC is urgent.

MicroRNAs (miRNAs) are highly conserved, small, noncoding, single-stranded RNAs with 19–24 nucleotides [6]. miRNAs transcriptionally or post-transcriptionally regulate gene expression through binding to targeted mRNAs and influence the degradation and translation of mRNA [6]. miRNAs regulate gene expression and the levels of proteins that act as oncogenes or cancer suppressors. Moreover, miRNAs are involved in various biological processes [7], and accumulating evidence suggests that aberrant levels of miRNAs are linked to proliferation, angiogenesis, and metastasis in various human malignancies [8]. With the development and application of molecular biology technology, a vital role for miRNAs in the diagnosis, prognosis, and therapy prediction of BC has been revealed [9–11]. The mature miRNA microRNA-101-3p (miR-101-3p, previously named miR-101) can be generated from miR-101-1 and miR-101-2 (the precursor miRNA, pre-miRNA). MiR-101-1 and miR-101-2 are located on different chromosomes and have different sequences, with diverse functions in the process of transcription [12]. Several studies have revealed that miR-101-3p is down-regulated in BC [13,14]. miR-101-3p inhibits proliferation, invasion, and metastasis via targeting Stathmin1 (STMN1) and CXCR7 [13,15] and promotes apoptosis by targeting JAK2 in BC cells [14]. Nevertheless, the precise mechanism of miR-101-3p in inhibiting neoplasia is still not entirely clear. Therefore, comprehensive analysis of the target gene networks and clinical value may help further clarify the function of miR-101-3p.

In recent years, the development of microarray technology has served as an effective measure to identify differentially expressed genes (DEGs) [16]. DEGs can be found through different experimental treatments, and their biological functions can be speculated via known information. Microarray technology has provided new insight into the alteration of gene expression during tumorigenesis [17]. Biomarkers associated with BC have been identified based on gene expression profiles. Several expression chips have confirmed the aberrant expression of miRNAs in BC and miRNAs influencing tumor behavior and progression [18,19]. A massive amount of complex biological information data has been generated and has greatly deepened our understanding of BC. Comprehensive analysis of gene expression patterns may aid in the prevention, treatment, and determination of prognosis in BC.

In this study, datasets of miR-101 in patients with BC, including 781 tumors and 87 adjacent non-tumor breast tissues from The Cancer Genome Atlas (TCGA), were explored. Furthermore, we analyzed the gene expression profile to identify DEGs between the miR-101-3p transfected group and the negative control group of BC cells. Bioinformatics analysis was carried out to predict targets of miR-101-3p. The target genes acquired from Gene Expression Omnibus (GEO) and prediction software were combined, and their potential roles were further explored with pathway, Gene Ontology (GO), and network analyses. The present study explored the comprehensive roles and prospective molecular mechanisms of miR-101-3p and might facilitate the discovery of potential novel biomarkers for future investigation of the mechanisms involved in BC.

Material and Methods

Patients in TCGA database

MiR-101 (miR-101-1 and miR-101-2) sequence data of BC were obtained from the TCGA dataset on 15 May 2016; the dataset included 781 patients with BC and 87 adjacent noncancerous breast tissues. In BC, the expression levels of log2-transformed miR-101-1 and miR-101-2 were analyzed, as was their correlation with survival data and clinical parameters.

Retrieval of BC gene expression microarray data

Microarray data for miR-101-3p transfected in BC cells were searched in Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/). The array data for GSE31397 were retrieved as raw data files based on the GPL570 platform (Affymetrix Human Genome U133 Plus 2.0 Array; Affymetrix, Inc., Santa Clara, CA). The alterations in miR-101-3p transfected MCF-7 cells compared with scramble control transfected cells were included in the expression profiling.

Data preprocessing and DEG analysis

The Affymetrix package [20] in R language preprocessed the original data, and probe-level data were mapped to gene names. The DEGs between the miR-101-3p transfected group and the control group were identified with the limma package of R language [21]. Down-regulated DEGs were determined with the criteria of fold change >2.5 and P value <0.05.
Clinical significance of miR-101 (miR-101-1 and miR-101-2) in BC based on TCGA data

The miR-101-2 expression level was lower in BC than in the normal cells, but this difference was not statistically significant (Figure 1A, 1D). A receiver operating characteristic (ROC) curve was constructed to identify diagnostic value in the cases of BC (Figure 1B). The AUC of miR-101-2 was 0.63 (95% CI: 0.58–0.68), with a sensitivity and specificity of 51.0% and 62.1%, respectively (Figure 1A, 1D). A receiver operating characteristic (ROC) curve was constructed to identify diagnostic value in the cases of BC (Table 2). Targets were acquired from the DGEs in GEO and the predicted genes that were identified in more than 5 out of 13 programs. Moreover, the experimental validation of the targets using a luciferase reporter assay, WB, and RT-PCR was collected from TarBase, mirTarBase, and published studies.

GO, KEGG and network analysis

To discern the biological attributes of the putative target genes, GO and KEGG enrichment analyses were completed using DAVID (https://david.ncifcrf.gov, version 6.7) [22]. The functional network graph of the selected genes was further visualized with Cytoscape 3.3.0 [23].

Statistical analysis

SPSS 20.0 was used for data analysis; data are presented as the mean ± standard deviation (SD). Statistical significance was assessed with a 2-sample t test between the cancer samples and adjacent noncancerous tissues and the correlation between miR-101-3p and clinical features. Survival data were determined with the Kaplan-Meier method. P<0.05 represented statistical significance.

Results

Clinical significance of miR-101 (miR-101-1 and miR-101-2) in BC based on TCGA data

The miR-101-2 expression level was lower in BC than in the normal cells, but this difference was not statistically significant (Figure 1A, 1D). A receiver operating characteristic (ROC) curve was constructed to identify diagnostic value in the cases of BC (Figure 1B). The AUC of miR-101-2 was 0.63 (95% CI: 0.58–0.68), with a sensitivity and specificity of 51.0% and 62.1%, respectively (Figure 1B). The AUC of miR-101-1 was 0.56 (95% CI: 0.50–0.62), with a sensitivity and specificity of 51.0% and 62.1%, respectively (Figure 1A). In the analysis of miR-101 and clinical parameters, miR-101-1 was prominently associated with the expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) in BC (Figure 1B). Moreover, miR-101-1 expression was more correlated with sex hormone receptors (ER, PR) and human epidermal growth factor receptor 2 (HER2) in BC (HR=1.79; 95% CI: 1.06–3.01; P=0.028) (Figure 1C). However, no significant prognostic value was identified for miR-101-2 in BC (HR=1.423, 95% CI: 0.84–2.41; P=0.183) (Figure 1F).

Potential targets of miR-101-3p in BC

As shown in Figure 2, GSE31397 array data were acquired, and 921 genes were selected as differentially expressed in miR-101-3p transfected MCF-7 cells compared with scramble control cells. In addition, the online prediction process was conducted with 13 databases to obtain potential targets of miR-101-3p, and 7924 genes were identified after duplicated genes were excluded. The genes predicted by 5 out of 13 programs were selected, and then 377 target genes were searched from the array data and predicted targets. Furthermore, 69 available genes that were identified as targets of miR-101-3p through TarBase, miTarBase and published literature were included (Table 3). Eventually, a total of 427 genes were identified for the gene-annotation enrichment analysis and KEGG pathway analysis.

Gene-annotation enrichment analysis and KEGG pathway analysis

Selecting Homo sapiens as the background of listed target genes in DAVID, we obtained the GO term annotations and KEGG pathway analysis through the functional annotation summaries. An EASE Score or modified Fisher’s exact test was utilized for calculating the p value. The results of the GO analysis are summarized in Table 4 and Figure 3, and the top 10 enriched items are listed according to p values. The biological processes (BP) of the potential targets of miR-101-3p markedly focused on the gene expression, transcription, metabolism, biosynthesis, and proliferation processes (p<0.001). As for the cellular component (CC), the target genes were significantly located in insoluble, cell, and membrane fractions (p<0.001). Moreover, molecular function (MF) category was enriched in transcription factors and proteins involved in nucleotide binding (p<0.001). In terms of the KEGG pathway analysis, the results were notably enriched in pathways involved in cancer, renal cell carcinoma, and colorectal cancer. Moreover, results were highly enriched in the VEGF, mTOR, focal adhesion, Wnt, chemokine, ErbB, and p53 signaling pathways (p<0.05, Table 5 and Figure 4). The enrichment graphs for the potential target genes were generated with Cytoscape software. As shown in Figure 5, the clearly associated functional modules were metabolism, development, cellular, and biological processes in BP. Analysis of CC revealed the most correlated functions were membrane and intracellular fractions (Figure 6). In the MF network, protein and transcription factor binding were the top results (Figure 7).
Figure 1. The clinical significance of miR-101 in BC in TCGA data. (A), miR-101-1 expression in BC compared with the normal group; (B), ROC curve analysis of miR-101-1 for discriminating BC from normal breast tissues; (C), Kaplan-Meier survival curves showed that lower miR-101-1 expression was associated with worse prognosis of patients with BC; (D), miR-101-2 expression in BC compared with the normal group; (E), ROC curve analysis of miR-101-2 for discriminating BC from normal breast tissues; (F), Kaplan-Meier survival curves revealed the connection between miR-101-2 and the prognosis of patients with BC.
### Table 1. Correlation of miR-101-1 expression with clinical parameters in BC of TCGA data.

| Clinicopathological features | Cases | MiR-101-1 expression | P value |
|------------------------------|-------|----------------------|---------|
|                              |       | Low | High |       |
| Age ≥50                      | 563   | 291 | 272  | 0.254 |
| Age <50                      | 218   | 99  | 119  | 0.110 |
| Gender Female                 | 772   | 382 | 390  | 0.241 |
| Gender Male                   | 9     | 8   | 1    |       |
| T T1–T2                       | 657   | 338 | 319  | 0.086 |
| T T3–T4                       | 123   | 52  | 71   |       |
| N N0                          | 373   | 183 | 190  |       |
| N N1–N3                      | 398   | 205 | 193  |       |
| M M0                          | 610   | 325 | 285  | 0.298 |
| M M1                          | 9     | 2   | 7    |       |
| Stage I–II                    | 587   | 290 | 297  | 0.782 |
| Stage III–IV                  | 186   | 94  | 92   |       |
| ER Positive                   | 565   | 259 | 306  | <0.001|
| ER Negative                   | 170   | 111 | 59   |       |
| PR Positive                   | 502   | 221 | 281  | <0.001|
| PR Negative                   | 231   | 148 | 83   |       |
| HER2 Positive                 | 99    | 57  | 42   | 0.037 |
| HER2 Negative                 | 397   | 202 | 195  |       |

T – tumor stage; N – lymph node stage; M – metastasis stage; ER – estrogen receptor; PR – progesterone receptor; HER2 – human epidermal growth factor receptor 2. The median was chosen as the cut-off value because of the skewed distribution of the expression data.

### Table 2. Correlation between miR-101-2 expression and clinical parameters in BC from the TCGA data.

| Clinicopathological features | Cases | MiR-101-2 expression | P value |
|------------------------------|-------|----------------------|---------|
|                              |       | Low | High |       |
| Age ≥50                      | 563   | 287 | 276  | 0.150 |
| Age <50                      | 218   | 104 | 114  | 0.110 |
| Gender Female                 | 772   | 385 | 387  | 0.485 |
| Gender Male                   | 9     | 6   | 3    |       |
| T T1–T2                       | 657   | 343 | 314  | 0.008 |
| T T3–T4                       | 123   | 48  | 75   |       |
| N N0                          | 373   | 200 | 173  |       |
| N N1–N3                      | 398   | 187 | 211  |       |
| M M0                          | 610   | 343 | 267  | 0.017 |
| M M1                          | 9     | 2   | 7    |       |
| Stage I–II                    | 587   | 298 | 289  | 0.129 |
| Stage III–IV                  | 186   | 88  | 92   |       |
| ER Negative                   | 170   | 88  | 82   |       |
| ER Positive                   | 502   | 245 | 257  | 0.250 |
| PR Negative                   | 231   | 118 | 113  |       |
| PR Positive                   | 99    | 54  | 55   | 0.481 |
| HER2 Negative                 | 397   | 207 | 190  |       |

T – tumor stage; N – lymph node stage; M – metastasis stage; ER – estrogen receptor; PR – progesterone receptor; HER2 – human epidermal growth factor receptor 2. The median was chosen as the cut-off value because of the skewed distribution of the expression data.
Figure 2. Flow chart showing target genes selection

Microarray data of miR-101-3p transfected in MCF-7 cells compared with scramble control was searched from Gene Expression Omnibus (GEO) (sample: GSE31397)

- Data processing and analysis
- Potential target genes of miR-101-3p predicted by DIANAmT, mirTarBase, RNA22, miRanda, PICTAR, miRDB, PolymiRTS, PITA, RNAhybrid, Targetscan, TargetMiner and TarBase databases (n=46,642)
- Genes of databases were merged and repeated genes were excluded
- Genes were merged and repeated genes were excluded
- Down-regulated differentially expressed genes (DEGs) were acquired (n=921)
- ≥5 repeated genes (n=2277)
- Total number of predicted genes (n=7924)
- Genes repeated more than 5 out of 13 programs were included
- Total number of target genes (n=377)
- Genes were merged and repeated genes were excluded
- Total included target genes (n=427)

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Total number of target genes (n=377)

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Total included target genes (n=427)

Table 3. Validated targets of miR-101-3p in TarBase, miTarBase and published literatures.

| Genes     | Validated in BC |
|-----------|----------------|
| ABCA1     |                |
| CFTR      |                |
| EZB2      |                |
| MCL1      |                |
| PTGS2     |                |
| SUZ12     |                |
| AMPK      |                |
| c-Met     |                |
| EZH2      |                |
| Mcl-1     |                |
| RAB5A     |                |
| TET2      |                |
| AP1       |                |
| COX2      |                |
| FBN2      |                |
| MEIS1     |                |
| Rac1      |                |
| TGFBR1    |                |
| APP       |                |
| CPEB1     |                |
| FMR1      |                |
| GCT1      |                |
| MET       |                |
| RAP1B     |                |
| VEGF      |                |
| ARID1A    |                |
| CXCL12    |                |
| FOS       |                |
| MITF      |                |
| RLI7P6    |                |
| VEGFA     |                |
| ATG4D     |                |
| CXCR7     |                |
| HMGA2     |                |
| MTO1      |                |
| ROCK2     |                |
| VEGF-C    |                |
| ATM       |                |
| DNMT3A    |                |
| JAK2      |                |
| MYCN      |                |
| RUNX1     |                |
| VHL       |                |
| ATP5B     |                |
| DUSP1     |                |
| KLF6      |                |
| NLK       |                |
| SOCS2     |                |
| ZEB1      |                |
| ATXN1     |                |
| EED       |                |
| Lin28B    |                |
| PIK3CB    |                |
| SOX9      |                |
| ZEB2      |                |
| CASP3     |                |
| EP4       |                |
| MAGI2     |                |
| Pim1      |                |
| SphK1     |                |
| CDH5      |                |
| EYA1      |                |
| MAPK1     |                |
| PRDM16    |                |
| STMN1     |                |
| CDK8      |                |
| EZB1      |                |
| MARCH7    |                |
| PTGER4    |                |
| Stmnl     |                |
| EYA1      |                |
| MAGI2     |                |
| Mcl-1     |                |
| VHL       |                |
### Table 4. GO functional annotation for the significant targets of miR-101-3p as determined by DAVID.

| Biological process                                                                 | GO ID      | GO term                                 | Count (%) | P value      | Gene symbol                                      |
|-----------------------------------------------------------------------------------|------------|-----------------------------------------|-----------|--------------|--------------------------------------------------|
| GO: 0010629 Negative regulation of gene expression                                | 006357     | Regulation of transcription from RNA polymerase ii promoter | 31 (0.4)  | $6.89 \times 10^{-6}$ | GCLC, THRB, CBX4, ZEB2, ZEB1, SOX9, PRDM16, LIN28B etc. |
| GO: 0010604 Positive regulation of macromolecule metabolic process                | 000668     | Positive regulation of macromolecule metabolic process | 43 (0.6)  | $1.36 \times 10^{-5}$ | GCLC, THRB, MITF, ZEB1, PRDM16, SOX9, FOS, APP, MEIS2 etc. |
| GO: 009891 Positive regulation of biosynthetic process                             | 006325     | Chromatin organization                   | 25 (0.4)  | $2.10 \times 10^{-5}$ | EZH2, NAP1L1, CBX4, CDYL, H2AFV, SMARC1D1 etc. |
| GO: 0051173 Positive regulation of nitrogen compound metabolic process             | 003128     | Positive regulation of cellular biosynthetic process | 35 (0.5)  | $2.15 \times 10^{-5}$ | GCLC, THRB, MITF, ZEB1, ABCA1, PRDM16, SOX9, FOS, APP etc. |
| GO: 008284 Positive regulation of cell proliferation                              | 0016568    | Chromatin modification                   | 20 (0.3)  | $4.74 \times 10^{-5}$ | DNMT3A, AEBP2, UBE2A, EZH2, CBX4, ARID1A, RBBP7, UBE2B etc. |
| GO: 002127 Regulation of cell proliferation                                         | 0005626    | Insoluble fraction                      | 43 (0.6)  | $3.83 \times 10^{-6}$ | GALNT3, GNAI3, PTGS2, PLXNA1, HMGCR, SGPP1, ADCY6 etc. |
| GO: 0000267 Cell fraction                                                          | 000624     | Membrane fraction                       | 40 (0.6)  | $2.65 \times 10^{-6}$ | GALNT3, GNAI3, PTGS2, PLXNA1, HMGCR, SGPP1, ADCY6 etc. |
| GO: 000694 Chromosome                                                               | 004427     | Chromosomal part                        | 25 (0.4)  | $4.29 \times 10^{-6}$ | CBX4, CDYL, SIN3A, H2AFV, PAFAH1B1, TOP2B, CHD6 etc. |
| GO: 000785 Chromatin                                                                | 009888     | Internal side of plasma membrane        | 21 (0.3)  | $2.14 \times 10^{-5}$ | RAP2C, RAB39B, AP1G1, MAOB, PIP5K1C, SNAPIN, CTNNA1 etc. |
| GO: 0019898 Extrinsic to membrane                                                  | 0019898    | Extrinsic to membrane                   | 27 (0.4)  | $3.26 \times 10^{-6}$ | PTGS2, CHMP5, ARHGAP17, MTRM2, DMXL2, PVR1, RAC1 etc. |
| GO: 0031974 Membrane-enclosed lumen                                                | 0043233    | Organelle lumen                         | 65 (0.9)  | $1.10 \times 10^{-6}$ | MRPL42, PTGS2, ZMAT3, ATP5B, EZH2, DNAJC10, ZEB1 etc. |
| GO: 008134 Transcription factor binding                                            | 000166     | Nucleotide binding                      | 85 (1.2)  | $7.96 \times 10^{-5}$ | CPEB2, HMGCR, ATP5B, UBE2G1, ADCY6, PIP5K1C, HELZ etc. |
Table 4 continued. GO functional annotation for the significant targets of miR-101-3p as determined by DAVID.

| GO ID          | GO term                              | Count (%) | P value          | Gene symbol                                                                 |
|----------------|--------------------------------------|-----------|------------------|------------------------------------------------------------------------------|
| GO: 0046332    | SMAD binding                         | 8 (0.1)   | 1.41×10^{-4}     | FOS, TGFBR1, ZEB2, SMAD2, SMAD1, PRDM16, PURB, PURA                           |
| GO: 0003690    | Double-stranded DNA binding          | 11 (0.2)  | 1.66×10^{-4}     | KLF6, FOS, ANKRD17, THR8, SMAD2, ZEB1, PURB etc.                            |
| GO: 0030528    | Transcription regulator activity      | 60 (0.9)  | 4.17×10^{-4}     | THR8, ELF5, EZH2, MTF, CBX4, ZEB2, ZEB1, LASS6, CBFA2T2 etc.                 |
| GO: 0003682    | Chromatin binding                     | 13 (0.2)  | 4.22×10^{-4}     | ATRX, SUZ12, DNMT3A, CDY1, EZH2, MTF, SMARCA5, CBX4 etc.                    |
| GO: 0003702    | RNA polymerase II transcription factor activity | 17 (0.2)  | 4.79×10^{-4}     | CEBPA, RXRB, MTF, TEAD3, SMAD1, SOX9, MED20, MEIS1 etc.                     |
| GO: 0032553    | Ribonucleotide binding                | 68 (1.0)  | 1.02×10^{-3}     | UBE2G1, ATP5B, ADCY6, PIP5K1C, HELZ, RAB1A, ANKRD17 etc.                    |
| GO: 0032555    | Purine ribonucleotide binding         | 68 (1.0)  | 1.02×10^{-3}     | UBE2G1, ATP5B, ADCY6, PIP5K1C, HELZ, RAB1A, ANKRD17 etc.                    |
| GO: 0043566    | Structure-specific DNA binding        | 12 (0.2)  | 1.13×10^{-3}     | KLF6, FOS, ANKRD17, THR8, SUB1, SMAD2, ZEB1, PURB etc.                      |

Figure 3. Functional annotation of the top 10 miR-101-3p targeted genes identified with GO using DAVID.
Table 5. KEGG pathway enrichment analysis of miR-101-3p target genes by DAVID.

| KEGG ID   | KEGG term                          | Count (%) | P value      | Gene symbol                                                                 |
|-----------|------------------------------------|-----------|--------------|------------------------------------------------------------------------------|
| hsa05200  | Pathways in cancer                 | 25 (0.4)  | 1.41×10⁻³   | PTGS2, MITF, FOS, CASP3, RAC1, TGFA, RUNX1, CEBPA, RXRB, PIK3CB, VHL, TGFBR1, MET, ITGA2, SMAD2, CDK6, CTNNA1, STK4, FZD4, FZD6, NRAS, MAPK1, CDKN1A, VEGFA, MTOR |
| hsa05211  | Renal cell carcinoma               | 10 (0.1)  | 1.44×10⁻⁴   | MAPK1, NRAS, VHL, PIK3CB, MET, VEGFA, GAB1, RAC1, TGFA, RAP1B                |
| hsa05210  | Colorectal cancer                  | 10 (0.1)  | 5.79×10⁻⁴   | MAPK1, FO5, PIK3CB, TGFBR1, MET, RAC1, SMAD2, FZD4, FZD6                   |
| hsa04520  | Adherens junction                  | 9 (0.1)   | 1.44×10⁻⁴   | PTPR1, MAPK1, PVR1, NLK, TGFBR1, MET, RAC1, SMAD2, CTNNA1                  |
| hsa05223  | Non-small cell lung cancer         | 7 (0.1)   | 4.05×10⁻³   | MAPK1, NRAS, PIK3CB, RXRB, TGFBR1, CDK6, STK4                                 |
| hsa05212  | Pancreatic cancer                  | 8 (0.1)   | 4.15×10⁻³   | MAPK1, PIK3CB, TGFBR1, VEGFA, RAC1, TGFA, CDK6, SMAD2                      |
| hsa05221  | Acute myeloid leukemia             | 7 (0.1)   | 5.79×10⁻⁴   | CEBPA, MAPK1, NRAS, PIK3CB, PIM1, MTOR, RUNX1                               |
| hsa05221  | Glioma                             | 7 (0.1)   | 8.65×10⁻³   | MAPK1, NRAS, CDKN1A, PIK3CB, TGFA, CDK6, MTOR                               |
| hsa04310  | Wnt signaling pathway              | 11 (0.16) | 1.06×10⁻²   | PSEN1, CCND3, VANGL1, ROCK2, NLK, RAC1, SMAD2, PLCB1, FBXW11, FZD4, FZD6  |
| hsa05218  | Melanoma                           | 7 (0.1)   | 1.52×10⁻²   | MAPK1, NRAS, CDKN1A, PIK3CB, MET, MITF, CDK6                                |
| hsa04150  | mTOR signaling pathway             | 6 (0.1)   | 1.56×10⁻²   | MAPK1, PIK3CB, VEGFA, PRKAA1, MTOR, DDIT4                                   |
| hsa04120  | Ubiquitin mediated proteolysis     | 10 (0.1)  | 1.57×10⁻²   | UBE2D3, FBXW1, UBE2A, VHL, UBE2G1, UBE2F, CUL4B, UBE2D1, FBXW11, UBE2B    |
| hsa04062  | Chemokine signaling pathway        | 12 (0.2)  | 1.71×10⁻²   | MAPK1, NRAS, GNAI3, ROCK2, GB1, PIK3CB, ADCY6, RAC1, RAP1B, JAK2, PLCB1, CXCL12 |
| hsa04670  | Leukocyte transendothelial migration | 9 (0.1) | 1.88×10⁻² | F11R, GNAI3, ROCK2, PIK3CB, RAC1, RAP1B, CTNNA1, CXCL12, CDH5 |
| hsa04370  | VEGF signaling pathway             | 7 (0.1)   | 1.94×10⁻²   | MAPK1, NRAS, PTGS2, PIK3CB, VEGFA, SPHK1, RAC1                                |
| hsa05220  | Chronic myeloid leukemia           | 7 (0.1)   | 1.94×10⁻²   | MAPK1, NRAS, CDKN1A, PIK3CB, TGFBR1, CDK6, RUNX1                            |
| hsa04916  | Melanogenesis                      | 8 (0.1)   | 2.22×10⁻²   | MAPK1, NRAS, GNAI3, MITF, ADCY6, PLCB1, FZD4, FZD6                          |
| hsa04510  | Focal adhesion                     | 12 (0.2)  | 2.76×10⁻²   | MAPK1, CCND3, ROCK2, PIK3CB, MET, VEGFA, RAC1, ITGA2, PIP5K1C, RAP1B, CAPN2, PARVA |
| hsa04360  | Axon guidance                      | 9 (0.1)   | 3.02×10⁻²   | MAPK1, NRAS, NRIP, GNAI3, PLXNA1, ROCK2, RAC1, MT, RAC1, CXCL12              |
| hsa04012  | ErbB signaling pathway             | 7 (0.1)   | 3.70×10⁻²   | MAPK1, NRAS, CDKN1A, PIK3CB, GAB1, MTOR                                   |
| hsa04115  | p53 signaling pathway              | 6 (0.1)   | 4.36×10⁻²   | CDKN1A, CASP3, CCND3, ZMAT3, CDK6, ATM                                     |
miRNAs play vital roles in BC, and an increasing amount of research has made important contributions to this field. MiRNAs, including miR-21/9/10b/27a/155, were overexpressed in BC, while miR-31/34a/125/205 were down-regulated during BC progression [24, 25]. Zhao et al. [26] demonstrated that miR-221 may be a predictive biomarker for BC. In the research of Eissa et al. [27], patients with BC positive for miR-10b had shorter relapse-free survival rates, and miR-10b was an independent prognostic factor of BC. MiR-451 influenced the drug resistances and miR-129-5p regulated radiosensitivity via accelerating the apoptosis of breast cancer cells [28, 29]. Peptide nucleic acid (PNA) has been researched as a novel drug in miR therapy, but has still not been successfully used to treat BC [30, 31]. Recently, decreased miR-101-3p levels were observed in tumors, including colon, gastric, lung, ovary, and prostate cancers [32]. miR-101-3p has important roles in the tumorigenesis of BC; however, the mechanisms and target genes of miR-101-3p are still unknown.

TCGA data demonstrated that miR-101-2 expression was lower in BC tissues than in normal tissues, while no statistical difference was shown for miR-101-1 expression. MiR-101-1 was also closely linked to ER, PR, and HER2, while miR-101-2 was associated with the T, N, and M stages of BC. This result revealed that miR-101-2 may have diagnostic value in BC to some extent. Down-regulated miR-101-1 was associated with poor prognosis in patients with BC, while no statistical significance was found for miR-101-2. miR-101 has 2 genomic loci, with miR-101-1 being located on chromosome 1p31.3 and miR-101-2 located on chromosome 9p24.1 [33]. This result revealed that the miR-101 transcripts on different chromosomes play diverse roles in the diagnosis, prognosis, and clinical outcome of BC. MiR-101-1 is processed into miR-101-3p and miR-101-5p, while miR-101-2 only produces mature miR-101-3p. In addition, the different sequences of miR-101-1 and miR-101-2 will promote or restrict different targets through participation in the translation process and reveal different biological functions [12]. However, it was difficult to extrapolate the mature miRNA levels (which are the final cellular effectors) based on these data. One precursor may be processed to 1 or 2 miRNAs; thus, the mature and precursor miRNA levels might not correlate, and this therefore will influence the clinical interpretation.

Putative miR-101-3p targets were derived from the expression profiling of miR-101-3p transfected in MCF-7 cells compared with scramble control cells, online prediction databases, validated targets, and published studies. Among the 427 putative target genes, the most predominant functions were transcription, metabolism, biosynthesis, proliferation, and transcription factor binding. This result indicated that candidate genes have a definitive impact on the pathogenesis of BC. In previous studies, 8 targets of miR-101-3p were validated in BC: AMP-activated protein kinase (AMPK), CX chemokine receptor 7 (CXCR7), eyes absent homolog 1 (EYA1), Janus kinase 2 (JAK2), membrane-associated guanylate kinase 2 (MAGI2), myeloid cell leukemia 1 (Mcl-1), Stathmin1 (STMN1), and von Hippel-Lindau tumor suppressor (VHL). AMPK was found to regulate tumor metabolism and be targeted by miR-101-3p and was identified as a promising therapeutic target in triple-negative breast cancer (TNBC) [34]. miR-101-3p inhibited the development and lymph node metastasis of BC via targeting CXCR7 [15]. The latest research by Guan et al. [35] showed that miR-101-3p was down-regulated and inhibited cell proliferation and promoted apoptosis by targeting EYA1 in BC. JAK2 has been verified to participate in suppressing proliferation and promoting apoptosis in BC cells through miR-101-3p [14]. Sachdeva et al. [36] demonstrated that miR-101-3p reduced...
Figure 5. The biological process (BP) network of miR-101-3p targeted genes was constructed using Cytoscape. The color and size of the nodes indicate the significance of the interactions.
The cellular component (CC) network of miR-101-3p targeted genes was constructed using Cytoscape. The color and size of the nodes indicate the significance of the interactions.

phosphatase and tensin homolog (PTEN) activity by suppressing MAGI-2, leading to Akt activation. MiR-101-3p, which directly inhibited MCL-1, was reported to restrain cell progression and increase sensitivity to paclitaxel in TNBC [37]. In a study by Wang et al. [13], the down-regulation of miR-101-3p was confirmed to regulate STMN1, and was associated with cellular proliferation and invasiveness in different subtypes of BC tissues. Moreover, miR-101-3p enhanced apoptosis and cell cycle arrest by down-regulating VHL expression in normoxic conditions [38].

Several studies have reported the signaling pathways associated with miR-101-3p in BC. miR-101-3p inhibited CXCR7-STAT3 signaling and exerted tumor-suppressive effects in BC cells [15]. Down-regulated miR-101-3p suppressed cell proliferation through the Notch signaling pathway in BC [34]. Furthermore, estrogen deprivation enhanced the miR-101-3p-mediated activation of the Akt signaling pathway [36]. In this paper, a total of 26 bio-pathways were identified, and analysis of miR-101-3p targets revealed statistical significance for 21 pathways. In addition, the visualized network graph of miR-101-3p-mediated targets in Cytoscape showed biological functions similar to the DAVID analysis mentioned above. The highly connected targets are involved in important biological processes and molecular functions.

Among the pathways of the miR-101-3p targeted genes, the VEGF, mTOR, focal adhesion, Wnt, chemokine, ErbB, and p53 signaling pathways were highly enriched. The VEGF signaling pathway enhanced angiogenesis for the aggressive proliferation and malignant progression of BC [39]. The PI3K/Akt/mTOR signaling pathway plays a vital regulatory function in proliferation, apoptosis, metabolism, and migration, and the invasion of BC can be alleviated by miR-199a-5p via targeting the FAK/Src/Akt/mTOR signaling pathway [40]. Focal adhesion kinase (FAK) regulates cell motility, extracellular matrix integrin signaling, cell proliferation, and survival. MiR-7 inhibited cell transformation and the metastasis of BC via regulating FAK, which is correlated with a poor prognosis [41]. The chemokine signaling pathway mediates chemokines and promotes

Figure 6. The cellular component (CC) network of miR-101-3p targeted genes was constructed using Cytoscape. The color and size of the nodes indicate the significance of the interactions.
Figure 7. The molecular function (MF) network of miR-101-3p targeted genes was constructed using Cytoscape. The color and size of the nodes indicate the significance of the interactions.
the chemotaxis, growth, and survival of BC cells [42]. miR-195 suppressed the Wnt signaling pathway, which promotes cell proliferation and the metastasis of TNBC [43]. Han et al. reported that the ErbB signaling pathway can be regulated by STAT1 in the tumorigenesis of BC [44]. Furthermore, scaffold/matrix-associated region-binding protein 1 (SMAR1) may increase radiosensitivity in the MCF-7 BC cell line by regulating the p53 signaling pathway [45]. Enrichment analysis of the potential pathways revealed that the Wnt signaling pathway probably regulates the cell cycle, metabolism, and phosphorylation by targeting CCND3 and ROCK. MAPK1, MTOR, and VEGFA may contribute to metabolism and biosynthesis, which are associated with the mTOR signaling pathway. The VEGF signaling pathway is involved in proliferation and apoptosis via targeting MAPK1, RAC1, NRAS, and VEGFA. The chemokine, focal adhesion, mTOR, VEGF, and ErbB signaling pathways might influence metabolism, phosphorylation, kinase activity, and intracellular signaling cascades by regulating PIK3CB. However, the interaction of target genes and their signaling pathways, as well as their molecular mechanisms, should be further explored.

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Conclusions

This study is a comprehensive target analysis of the miR-101 using gene expression profiling, public databases, and prediction software. MiR-101 plays vital roles in BC, while the products derived from different locations on different chromosomes did not show the same functions. The genes targeted by miR-101-3p influence the progression of BC and might influence the biological functions performed by the VEGF, mTOR, focal adhesion, Wnt, and chemokine signaling pathways by targeting crucial genes. The mechanisms of novel molecular markers should be further verified with new techniques, and will contribute to the diagnosis and treatment of BC.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.
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