Abstract. Previous research has proven that 6-thioguanine (6-TG) inhibits the growth of MCF-7 breast cancer cells. Accumulating evidence indicates that long non-coding (lnc) RNAs are involved in the development of various cancer types as competitive endogenous (ce)RNA molecules. The present study was conducted to investigate the regulatory mechanism underlying the function of lncRNAs as ceRNA molecules in MCF-7 cells and to identify more effective prognostic biomarkers for breast cancer treatment. The expression profiles of lncRNAs in untreated MCF-7 cells and 6-TG-treated MCF-7 cells were compared by RNA-seq. The regulatory associations among lncRNAs, micro (mi)RNAs and mRNAs were analyzed and verified by the TargetScan, miRDB and miRTarBas databases. The ceRNA networks were constructed by Cytoscape. The expression levels of two lncRNAs and two miRNAs in the ceRNA network were measured by reverse transcription-quantitative PCR. The OncoLnc and Kaplan-Meier plotter network databases were utilized to determine the effects of lncRNA and miRNA expression on the survival of patients with breast cancer. A ceRNA network was constructed for MCF-7 breast cancer cells treated with 6-TG, and this network may provide valuable information for further research elucidating the molecular mechanism underlying the effects of 6-TG on breast cancer. Moreover, LINC00324, MIR22HG, miR-370-3p and miR-424-5p were identified as potential prognostic and therapeutic biomarkers for breast cancer.

Introduction

Breast cancer is one of the most common malignancies and is the most common cancer in women. In 2018, 2.1 million new cases of breast cancer were diagnosed worldwide (1). Approximately 75% of breast cancer is luminal subtype (ER+), and the number of young women with estrogen receptor-positive breast cancer has gradually increased (2). Currently, surgery and chemotherapy are the primary treatment methods for breast cancer, and medical oncology has been employed to treat early-stage breast cancer, but treatments for breast cancer remain expensive, especially in developing countries. In addition, due to the high frequency of metastasis and drug resistance in breast cancer, the recurrence and mortality rate of breast cancer are increasing rapidly (3). Therefore, it is urgently important to elucidate the molecular pathogenesis of breast cancer and identify new candidate therapeutic targets and biomarkers for this disease to improve breast cancer detection and treatment.

Many standard clinical drugs, such as cyclophosphamide, capecitabine and doxorubicin, have been determined to have inhibitory effects on breast cancer (4-7). 6-TG has been used as an anticancer and immunosuppressant in medical practice for over 50 years (8). Initially, 6-TG was only employed to treat acute and chronic myeloid leukemia (9). Following further research, 6-TG has proven to have therapeutic effects on breast cancer, and medical oncology has been employed to treat early-stage breast cancer, but treatments for breast cancer remain expensive, especially in developing countries. In addition, due to the high frequency of metastasis and drug resistance in breast cancer, the recurrence and mortality rate of breast cancer are increasing rapidly (3). Therefore, it is urgently important to elucidate the molecular pathogenesis of breast cancer and identify new candidate therapeutic targets and biomarkers for this disease to improve breast cancer detection and treatment.

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RNAs, and other RNAs lacking conservation across species, such as long non-coding (linc)RNAs, which constitute 60% of transcripational outputs in human cells (13). These ncRNAs have gradually been associated with human diseases and especially play important roles in the occurrence and development of cancer (14). Numerous studies have shown that IncRNAs, as ceRNAs, are involved in the occurrence and development of a variety of cancer types, such as esophageal, breast, liver and pancreatic cancer (15-18). The ceRNA was proposed as a novel regulatory mechanism between ncRNA and coding RNA (19). The types of ceRNAs include mRNAs, IncRNAs and circRNAs. These ceRNAs act as molecular sponges of miRNAs through their MREs, thereby regulating the target genes of the corresponding miRNAs (20). Complex crosstalk of the ceRNA network has been observed in different cancer types (21). Wang et al (22) selected 322 muscle-invasive bladder cancer tissues and 19 non-tumour bladder tissues from The Cancer Genome Atlas (TCGA) and further analysed the RNA profiles in these tissues. Next, a lncRNA-associated ceRNA network was constructed, and novel lncRNAs were identified as candidate prognostic biomarkers for muscle-invasive bladder cancer. Zheng et al (23) constructed a STARD13-correlated ceRNA network to explore the effects of this network on breast cancer stemness and demonstrated that activation of the STARD13-correlated ceRNA network was accompanied by inhibition of the activity of YAP/TAZ in regulating breast cancer stem cell traits. Fan et al (24) analyzed RNA sequencing data of patients with breast cancer from TCGA database and established a lncRNA-miRNA-mRNA ceRNA network. Finally, four lncRNA biomarkers were identified as prognostic signatures in breast cancer. Taken together, the findings of these studies show that dysregulated IncRNAs in the ceRNA network can directly interact by causing miRNA sponges to regulate mRNA expression, thereby contributing to cancer initiation and progression (25). Nevertheless, there are significant differences among the breast cancer subtypes regarding their molecular profiles and responses to therapy, and very little information is available on breast cancer ceRNAs.

A previous study determined that 6-TG can inhibit the proliferation of the MCF-7 breast cancer cell line. The changes in the ceRNA network that may be caused by 6-TG through the mediation of lncRNAs requires further exploration. In the present study, differentially expressed IncRNAs and mRNAs were determined by RNA-seq in untreated MCF-7 cells and 6-TG-treated MCF-7 cells and predicted their common interactional miRNAs. Subsequently, the lncRNA-miRNA-mRNA ceRNA network was successfully constructed by comparing, predicting and integrating differentially expressed RNAs. This study elucidated the lncRNA-mediated ceRNA regulatory mechanism underlying the effects of 6-TG treatment on MCF-7 breast cancer cells and identified potential diagnostic biomarkers that may contribute to the early diagnosis, treatment and prognosis of luminal subtype breast cancer.

Materials and methods

Cell culture and cell viability assay. The breast cancer cell line MCF-7 was purchased from the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). MCF-7 cells were maintained at 37˚C in a cell incubator with 5% CO₂. The cells were cultured in high-glucose DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Biological Industries), 1% MEM nonessential amino acids (Gibco; Thermo Fisher Scientific, Inc.), 1% GluMAX and 1% penicillin-streptomycin solution (Gibco; Thermo Fisher Scientific, Inc.). The effects of 6-TG on cell viability were determined through the Cell Counting Kit-8 assay, and MCF-7 cells were treated with a half-maximal inhibitory concentration of 6-TG (12).

Data processing and differential expression analysis. The sequencing data of samples from the MCF-7 control and 6-TG groups were obtained from a previous study (12). The raw data were available from the NCBI GEO database (https://www.ncbi.nlm.nih.gov/geo/) (GSE130161) (12). The differential expression of IncRNAs were confirmed by performing normalization and log₂ conversion. The IncRNAs with log₂FC≥2 and P<0.05 were selected as candidate lncRNAs.

Weighted gene correlation network analysis (WGCNA). A WGCNA network was generated for several subsets of the data. The WGCNA package (v1.67) (https://github.com/paytonyau/WGCNA) was installed for co-expression analysis using BiocManager (http://bioconductor.org/biocLite.R). The soft threshold method was employed to carry out Pearson correlation analysis on the expression profiles to determine the connection strength between two genes, thereby constructing a weighted network. Based on the topological overlap difference of network connection strength, average link hierarchical clustering was performed on group transcripts. To obtain the correct module number and clarify gene interactions, the restricted minimum gene number was set to 5 for each module, and a threshold of 0.25 was used to merge the similar modules. Next, the significant differentially expressed IncRNAs (sDEs) were collaborated with the hub genes of candidate pathways in the module that were considered to be potential targets for the therapeutic mechanism of 6-TG.

Prediction of lncRNA-miRNA and miRNA-mRNA interactions. To predict the interaction of lncRNA and miRNA, the data were downloaded from the starBase V2.0 database (http://starbase.sysu.edu.cn/). In addition, the miRNA target prediction online tools Targetscan7.1 (http://www.targetscan.org/), miRDB (http://www.mirdb.org/miRDB/) and miRTarBase (http://miirtarbase.mbc.nctu.edu.tw) were employed to predict the miRNA of mRNA. Finally, a Venn diagram analysis through TBtools (https://github.com/CJ-Chen/TBtools/releases) was used to obtain the common target miRNAs of differential expression IncRNAs and mRNAs.

Construction of the lncRNA-miRNA-mRNA ceRNA network. Cytoscape (www.cytoscape.org/) is a visual source software platform for constructing molecular interaction networks and biological pathways and integrating these networks with annotation, gene expression profiles, and other data (26). Based on the associations among differentially expressed IncRNAs, miRNAs and mRNAs, Cytoscape3.7.2 was employed to construct and visualize the lncRNA-miRNA-mRNA ceRNA network, where the nodes
represented mRNAs, lncRNAs or miRNAs and the edges represented their interactions.

RNA isolation and reverse transcription-quantitative (RT-q) PCR verification. Total RNA was extracted from MCF-7 cells using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer’s protocol. For the lncRNA verification, reverse transcription reactions with Transcriptor All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (TRAN, China) were performed in two steps: 15 min at 42˚C and 5 sec at 85˚C. The qPCR assay was performed in 96-well optical reaction plates using SYBR® Premix Ex Taq™ reagents (Takara Biotechnology Co., Ltd.) and a Light Cycle® 96 Real-Time PCR System (Roche Diagnostics). The qPCR steps were as follows: 30 sec denaturation at 95˚C, 45 cycles of PCR for the quantitative analysis (95˚C for 5 sec and 60˚C for 30 sec), one cycle for the melting curve analysis (95˚C for 5 sec, 60˚C for 1 min, and 95˚C for 1 sec) and cooling at 4˚C. For miRNA verification, reverse transcription reactions using the miRcute Plus miRNA First-Strand cDNA kit (Tiangen Biotech Co., Ltd.) were performed in two steps: 60 min at 42˚C and 3 min at 95˚C. The qPCR assay was performed in 96-well optical reaction plates using SYBR® Premix Ex Taq™ reagents (Takara Biotechnology Co., Ltd.) and a Light Cycle® 96 Real-Time PCR System (Roche Diagnostics). The qPCR steps were as follows: 15 min denaturation at 95˚C, 45 cycles of PCR for the quantitative analysis (94˚C for 20 sec and 60˚C for 34 sec), one cycle for the melting curve analysis (95˚C for 5 sec, 60˚C for 1 min, and 95˚C for 1 sec) and cooling at 4˚C. The primer sequences of lncRNAs were as follows: MIR22HG-forward (F), TGGCTGTGGGACAGTGATGAG; MIR22HG-reverse (R), GCGAGGGCTGGAGGGAGATG; LINC00324-F, GGGTTGGGACTTGGAGC; LINC00324-R, AC005786.5 ENSG00000189275 2.0108714038 0.00298 Up LINC02672 ENSG00000227121 2.032007338 0.00365 Up RP11763B22.6 ENSG00000235887 2.029273852 0.0112 Up

Survival analysis of lncRNAs and miRNAs. In order to assess the association between days and surviving percentages of clinical patients, data regarding the expression of lncRNAs and miRNAs and the survival of patients with cancer were analyzed by Cox regression analysis of survival packages and Kaplan-Meier curves on the OncoLnc (https://www.oncolnc.org) and Kaplan-Meier-plotter (Breast Cancer) (http://kmplot.com/analysis/).

Statistical analysis. All data are presented as the mean ± SD, and the qPCR data were analyzed with Prism 7 (GraphPad Software, Inc.) by the unpaired t-test. P<0.05 was considered to indicate a statistically significant difference. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Results

Inhibitory effect of 6-TG on MCF-7 cells and identification of differentially expressed lncRNAs. The cell proliferation
The cell proliferation activity of the 6-TG treatment group was significantly decreased compared with the control group (Fig. 1A). Morphological changes of the MCF-7 cells were observed after 6-TG treatment. The untreated control group had well-proliferated cells, while the 6-TG-treated group showed morphological changes such as cell shrinkage, nuclear contraction and fragmentation, and disrupted intercellular connections (Fig. 1B). mRNA and lncRNA expression profiles were identified between the control and 6-TG treatment groups. The volcano plot revealed 1382 upregulated and 597 downregulated mRNAs (Fig. 1C). A total of 1621 differentially expressed lncRNAs were identified, including 1463 upregulated and 158 downregulated lncRNAs (Fig. 1D). Using |logFC|>2.0 and P<0.05 as criteria, 21 differentially expressed lncRNAs were screened out (Table I). To determine whether the identified co-expression modules were associated with 6-TG treatment in the MCF-7 cells, WGCNA was performed with the mRNA samples, and all significant differentially expressed genes (sDEGs) were merged into five modules according to the degree of co-expression across the WGCNA dataset. Gene dendrogram analysis showed that the turquoise module contained the most co-expressed genes, and it contained 795 genes in total. The integrated heatmap further showed that these genes were upregulated, accounting for more than half of the total upregulated genes, and they were the major groups responding to 6-TG (Fig. 1E). Pathway enrichment analysis showed that only the genes in the turquoise module could be enriched in the Wikipedia pathway database. The enrichment analysis revealed that the cell cycle pathway was the most enriched, while...
the enrichment degree of the apoptosis pathway was the most significant. The regulation of sister chromatid separation at the metaphase-anaphase transition, TP53 network and gastric cancer network were the three pathways with the highest enrichment ratio (Fig. 1F).

Matching of lncRNAs-miRNAs and prediction of mRNAs-miRNA interaction. Based on the online database of StarBase V2.0, the lncRNA-miRNA pairs were predicted and seven candidate lncRNAs and 125 miRNAs were compared. Both pathway enrichment and the results of a previous study (12) demonstrated that apoptosis and the p53 signaling pathway were the two main pathways induced by 6-TG in MCF-7 cells, and the aberrant expression of mRNAs from these two pathways is depicted in Table II. Next, the target miRNAs of these differentially expressed mRNAs were predicted using TargetScan, miRDB and miRTarBas online software. Finally, 4 lncRNAs, 10 miRNAs and 25 mRNAs were successfully matched though StarBase verification. The results of this analysis are shown in Table III.

Construction of lncRNA-miRNA-mRNA ceRNA network. Cytoscape 3.7.2 was used to visualize the lncRNA-miRNA-mRNA ceRNA network. Based on the aforementioned data, the lncRNA-miRNA-mRNA ceRNA network was constructed. As shown Fig. 2A, 4 lncRNAs, 10 miRNAs and 25 mRNAs were involved in the ceRNA network. According to the network, has-miR-370-3p (degree, 20) and has-miR-424-5p (degree, 9) were identified as hub regulatory elements. Therefore, a more meaningful ceRNA network was obtained based on the MIR22HG-has-miR-370-3p and LINC0034-has-miR-424-5p pairs (Fig. 2B). This core ceRNA network included several hub genes, such as FAS, CCND3 and CDKN1A, which played important roles in 6-TG-induced apoptosis in MCF-7 cells (12).

RT-qPCR verification and overall survival assessment of specific lncRNAs and miRNAs. To validate the reliability and validity of the aforementioned analysis data, RT-qPCR was applied to evaluate the differences in the expression of main regulatory molecules between the control and 6-TG treatment groups. The results showed that has-miR-370-3p and has-miR-424-5p were downregulated in 6-TG treatment group compared with the control group, while MIR22HG and LINC0034 were upregulated in the 6-TG treatment group (Fig. 3A and B). These results were in keeping with the bioinformatic results. The lncRNAs, such as MIR22HG and LINC0034, may regulate the activities of hub genes by sponging the target miRNAs (miR-370-3p and miR-424-5p). According to the clinical patients’ survival rates, the two specific lncRNAs and two specific miRNAs from the ceRNA network were further analyzed. High expression of LINC00324 and MIR22HG was associated with high survival rates. Regarding LINC00324, the survival rates of high- and low-expression groups were

Table II. Differentially expressed mRNA of p53 signaling pathway and apoptosis pathway.

| p53 signaling pathway mRNA | Apoptosis pathway mRNA |
|---------------------------|------------------------|
| STEAP3, ZMAT3, RRM2B      | XIAP, NFKBIA, NFKB1    |
| CCNG1, SESN2, CCNG2       | AKT1, ACTG1, FOS       |
| SESN1, CDKN1A, E2F4       | TNFRSF1A, PIK3CA, DIABLO |
| TP53F3, PPM1D, CCND3      | TUBA1A, PIK3R3, TUBA1B |
| TSC2, DDB2, MDM2          | AKT2, PIK3R2, ACTB     |
| IGFBP3, BBC3, BAX         | CFLAR, MAP2K2, RELA    |
| GADD45G, TP53, PIDD1      | CAPN2, DDIT3, CAPN1    |
| FAS, GADD45A              | MAPK1, TNFRSF10C, CT5K |
|                          | JUN, IKBKG, MAPK3      |
|                          | IKBKB, IL3RA, SPTAN1   |
|                          | CT5F, TNFRSF10B, BBC3  |
|                          | AX, GADD45G, TP53      |
|                          | PIDD1, FAS, GADD45A    |

Figure 2. lncRNA-miRNA-mRNA ceRNA Network. (A) lncRNA-miRNA-mRNA ceRNA Network: Triangles represent lncRNA; rhombuses represent miRNA; circles represent mRNA. (B) Core ceRNA network: Triangles represent lncRNA; rhombuses represent miRNA; circles represent mRNA. lncRNA, long non-coding RNA; miRNA, microRNA; ceRNA, competitive endogenous RNA.
In the present study, screening criteria were used to select differentially expressed lncRNAs. In previous studies, the criteria for screening was |logFC|≥2.0 and P<0.05. However, in this study, the criteria were |logFC|>1.0 and P<0.05. With these criteria, 10 miRNAs and 25 mRNAs were successfully constructed by bioinformatic technology. The ceRNA network included 4 lncRNAs, 18 miRNAs and 26 mRNAs from invasive breast cancer samples and 90 lncRNAs, 18 miRNAs and 26 mRNAs from invasive breast cancer samples. In contrast, PURPL regulated the growth and proliferation of liver cancer cells and promoted the proliferation of liver cancer cells. A previous study showed that PURPL regulated the growth of cancer cells by inhibiting the mutual binding of p53 and the p53-activating protein MYBBP1A in colon cancer. A study in liver cancer also showed that PURPL and p53 mRNA expression levels were negatively correlated in hepatocellular carcinoma cells and promoted the proliferation of liver cancer cells.

### Discussion

In recent years, the role played by lncRNA as a ceRNA has become a highly studied topic in the field of tumor research. Prior studies indicated that the lncRNA-miRNA-mRNA ceRNA network was involved in the development of lung cancer, esophageal cancer and gastric cancer. However, as the classification of breast cancer is complicated and the response of this cancer to treatment is highly variable, further research is warranted to determine the role played by ceRNA in breast cancer.

In the field of breast cancer ceRNA research, Jia et al (26) constructed a ceRNA network consisting of 44 miRNA-lncRNA interaction pairs and two miRNA-mRNA interaction pairs, based on the information from 428 HR+/Her-2- and 113 triple-negative breast cancer samples from TCGA. These researchers selected SFRP1, AC006449.1 and MUC2 as novel clinical targets of breast cancer. Gao et al (26) constructed a ceRNA network including 90 lncRNAs, 18 miRNAs and 26 mRNAs from invasive breast cancer samples and found that LINC00466, hsa-mir-204 and NTRK2 were associated with the prognosis of breast cancer. In the present study, by RNA-seq, differentially expressed lncRNAs and mRNAs were obtained from MCF-7 breast cancer cells with and without 6-TG treatment, and their common target miRNAs were predicted. A ceRNA network consisting of 4 lncRNAs, 18 miRNAs and 26 mRNAs from invasive breast cancer samples and 90 lncRNAs, 18 miRNAs and 26 mRNAs from invasive breast cancer samples was constructed and the regulatory mechanism underlying the role played by lncRNA as ceRNA in MCF-7 breast cancer cells treated with 6-TG was elucidated.

Prior studies indicated that the lncRNA-miRNA-mRNA ceRNA network was involved in the development of lung cancer, esophageal cancer and gastric cancer (27-29). However, as the classification of breast cancer is complicated and the response of this cancer to treatment is highly variable, further research is warranted to determine the role played by ceRNA in breast cancer. Therefore, it is important to study the differential expression of lncRNA, miRNA and mRNA in breast cancer and characterize their regulatory association to identify potential biomarkers and new drug targets for the diagnosis, prognosis and treatment of breast cancer. In the present study, RNA-seq and bioinformatic technology were employed to analyze the differentially expressed lncRNA, the ceRNA network was constructed and the regulatory mechanism underlying the role played by lncRNA as ceRNA in MCF-7 breast cancer cells treated with 6-TG was elucidated.

| LncRNA-ID | miRNA-ID | mRNA-ID |
|-----------|-----------|---------|
| LINC01176 | hsa-miR-155-5p | FOS |
| LINC01176 | hsa-miR-155-5p | PIK3CA |
| LINC00324 | hsa-miR-15b-5p | CCND3 |
| LINC00324 | hsa-miR-15b-5p | ZMAT3 |
| LINC00324 | hsa-miR-15b-5p | PPM1D |
| MIR22HG   | hsa-miR-24-3p | ZMAT3 |
| MIR22HG   | hsa-miR-24-3p | PIK3R3 |
| MIR22HG   | hsa-miR-24-3p | SESN1 |
| LINC00324 | hsa-miR-479-5p | CCND3 |
| LINC00324 | hsa-miR-479-5p | GADD45G |
| LINC00324 | hsa-miR-6838-5p | SESN2 |
| LINC00324 | hsa-miR-6838-5p | ZMAT3 |
| LINC00324 | hsa-miR-6838-5p | SESN1 |
| MIR22HG   | hsa-miR-6893-3p | CDKN1A |
| PURPL     | hsa-miR-19a-3p | FAS |
| PURPL     | hsa-miR-19a-3p | TP53 |
| PURPL     | hsa-miR-19a-3p | MAPK1 |
| PURPL     | hsa-miR-19a-3p | ZMAT3 |
| PURPL     | hsa-miR-19a-3p | PIK3R3 |
| LINC00324 | hsa-miR-424-5p | IGFBP3 |
| LINC00324 | hsa-miR-424-5p | CCND3 |
| LINC00324 | hsa-miR-424-5p | PPM1D |
| LINC00324 | hsa-miR-424-5p | ZMAT3 |
| LINC00324 | hsa-miR-424-5p | GADD45G |
| LINC00324 | hsa-miR-424-5p | TUBA1A |
| LINC00324 | hsa-miR-424-5p | IKKB |
| LINC00324 | hsa-miR-424-5p | SESN2 |
| LINC00324 | hsa-miR-424-5p | SESN1 |
| MIR22HG   | hsa-miR-370-3p | CDKN1A |
| MIR22HG   | hsa-miR-370-3p | GADD45A |
| MIR22HG   | hsa-miR-370-3p | STEAP3 |
| MIR22HG   | hsa-miR-370-3p | MAP2K2 |
| MIR22HG   | hsa-miR-370-3p | SESN2 |
| MIR22HG   | hsa-miR-370-3p | AKT1 |
| MIR22HG   | hsa-miR-370-3p | MAPK1 |
| MIR22HG   | hsa-miR-370-3p | FOS |
| MIR22HG   | hsa-miR-370-3p | TNFRSF1A |
| MIR22HG   | hsa-miR-370-3p | CCND3 |
| MIR22HG   | hsa-miR-370-3p | JUN |
| MIR22HG   | hsa-miR-370-3p | IKKB |
| MIR22HG   | hsa-miR-370-3p | GADD45G |
| MIR22HG   | hsa-miR-370-3p | PIK3CA |
| MIR22HG   | hsa-miR-370-3p | MDM2 |
| MIR22HG   | hsa-miR-370-3p | FAS |
| MIR22HG   | hsa-miR-370-3p | PIK3R3 |
| MIR22HG   | hsa-miR-370-3p | CTSF |
| MIR22HG   | hsa-miR-370-3p | AKT2 |

IncRNA, long non-coding RNA; miRNA, microRNA.
cells by regulating p53 expression (33). In the present study, PURPL was also screened by the integrated bioinformatic analyses, which indicated that this lncRNA played an important role in multiple tumors, including breast cancer. However, the present results showed that PURPL was highly expressed and positively correlated with p53 expression in MCF-7 cells after the 6-TG treatment. It was hypothesized that PURPL might act as ceRNA by inhibiting its targeted miR-19a-3p to regulate p53 expression and induce apoptosis in MCF-7 cells. Prior studies showed that LINC00324 acted as a ceRNA to regulate AKT1 expression by inhibiting its targeted miR-615-5p in lung adenocarcinomas, and LINC00324 promoted gastric cancer cell proliferation by binding to RNA binding protein HuR to regulate FAM83B protein expression in gastric cancer (34,35). Ni et al (36) measured the expression level of LINC00324 and miR-214-3p in CRC cells by RT-qPCR and proved that LINC00324 regulated CRC cell proliferation by sponging miR-214-3p. In the present study, LINC00324 was selected as a hub lncRNA in the ceRNA network and was observed to target miR-424-5p to regulate downstream mRNA. In the present study, LINC00324 was selected as a hub lncRNA in the ceRNA network and was observed to target miR-424-5p to regulate downstream mRNA. Meanwhile, the results of qPCR analysis verified that the expression of LINC00324 was increased, while the expression of miR-424-5p was decreased, in MCF-7 cells after 6-TG treatment. Therefore, LINC00324 may inhibit the proliferation and induce apoptosis of MCF-7 cells by sponging miR-424-5p to exert ceRNA regulation in the breast cancer cells. The function of MIR22HG as a tumor suppressor has been confirmed in basic research of liver, lung and thyroid cancer, and it plays a key role in the progression of many tumors by inhibiting tumor cell proliferation, invasion and metastasis (37-39). Wu et al (40) verified that knockdown of MIR22HG promoted growth, migration and invasion of hepatocellular carcinoma cells (HCC) and demonstrated that MIR22HG functioned as a ceRNA to inhibit the Wnt/β-catenin pathway, by sponging miR-10a-5p in the HCC. Su et al (39) reported that MIR22HG was downregulated in lung cancer and suggested that silencing of MIR22HG increased lung cancer cells proliferation, migration and invasion. However, to the best of our knowledge, no relevant research has been conducted in breast cancer. The results of the present study indicated that MIR22HG, which is highly expressed in MCF-7 cells treated with 6-TG, served as a hub lncRNA to regulate downstream target genes by targeted inhibition of miR-370-3p to induced apoptosis. Therefore, MIR22HG is likely to be a potential therapeutic target for breast cancer.

The miRNA is the core factor of the ceRNA network and is an important tumor marker. Zhang et al (41) indicated that high expression of miR-424-5p increased the proliferation and invasion of gastric cancer cells. Liu et al (42) also found that miR-424-5p promoted lung metastasis ability of thyroid cancer cells in lung colonization models in vivo. Therefore, miR-424-5p served as an oncogene in several cancer types such as gastric, thyroid and colorectal cancer (41-43).
Hou et al (44) reported that miR-370-3p expression was increased in cerebral aneurysm tissues compared with normal tissues, and miR-370-3p was involved in the development of cerebral aneurysm by targeting the KDR/AKT signaling pathway. Lyu et al (45) suggested that miR-370-3p, as a core molecular, played important role in breast cancer. The pseudogene HLA-DPB2 upregulated HLA-DPB1 through sponging miR-370-3p to exert antitumor effect by recruiting tumor-infiltrating immune cells into the breast tumor microenvironment. In the present study, miR-424-5p and miR-370-3p were identified as hub regulatory elements in the ceRNA network, and the qPCR results showed that the expression levels of these two key miRNAs were decreased in MCF-7 cells after 6-TG treatment. The results indicated that miR-424-5p and miR-370-3p were also crucial biomarkers in breast cancer. Furthermore, miRNAs affected gene expression primarily by regulating targeted mRNAs. GADD45G has been implicated in a variety of processes, such as apoptosis, DNA repair and cell cycle checkpoints (46). The previous studies (47,48) showed that GADD45G inhibited the migration of esophageal squamous cell carcinoma (ESCC) cells and was associated with ESCC patients' survival. CCND3 has been verified to regulate cell cycle progression. It was confirmed that CCND3 could bind to CDKs and formed complex compounds, and the upregulation of cyclin D-CDK compounds was observed to inhibit cell cycle progression in multiple myeloma (49). In our ceRNA network, GADD45G and CCND3, as the terminal effector molecule, were commonly targeted mRNA by both miR-424-5p and miR-370-3p. Meanwhile, pathway enrichment analysis indicated that differential expression mRNAs were mainly enriched in the cell cycle and apoptosis pathway. Therefore, GADD45G and CCND3 might play important roles in apoptosis and cell cycle regulation of breast cancer.

In conclusion, a ceRNA network was constructed based on the screening criteria of |logFC|≥2.0 and P≤0.05 and used bioinformatics to analyze the regulatory mechanism by which IncRNAs function as ceRNA molecules in MCF-7 breast cancer cells. Differentially expressed IncRNAs with potential research value were obtained, such as MIR22HG and LINC00324, which may provide new prognostic biomarkers for the treatment of breast cancer. The present study helps to establish a theoretical basis for further research elucidating the molecular mechanism of breast cancer regulation and screening potential therapeutic targets.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HL, ZL and HY conducted the analyses and interpreted the data. QL and XA performed the experiments. HL was a major contributor in designing the study and writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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