

OPEN ACCESS

EDITED BY

Yiran Liang,
Shandong University, China

REVIEWED BY

Xiaojin Song,
Shandong University, China
Haizhen Lu,
Chinese Academy of Medical Sciences, China

*CORRESPONDENCE

Guangdong Qiao
qiaogddy@163.com
Yizi Cong
congyizi@163.com

[†]These authors have contributed equally to this work

SPECIALTY SECTION

This article was submitted to Surgical Oncology, a section of the journal Frontiers in Surgery

RECEIVED 17 August 2022

ACCEPTED 24 November 2022

PUBLISHED 06 January 2023

CITATION

Chen G, Li Y, Cao J, Dai Y, Cong Y and Qiao G (2023) Construction and analysis of a survival-associated competing endogenous RNA network in breast cancer. *Front. Surg.* 9:1021195. doi: 10.3389/fsurg.2022.1021195

COPYRIGHT

© 2023 Chen, Li, Cao, Dai, Cong and Qiao. This is an open-access article distributed under the terms of the [Creative Commons Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Construction and analysis of a survival-associated competing endogenous RNA network in breast cancer

Gang Chen^{1†}, Yalun Li^{1†}, Jianqiao Cao¹, Yuanping Dai², Yizi Cong^{1*} and Guangdong Qiao^{1*}

¹Department of Breast Surgery, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai, China, ²Department of Medical Genetics, Liuzhou Maternal and Child Health Hospital, Liuzhou, China

Background: Recently, increasing studies have shown that non-coding RNAs are closely associated with the progression and metastasis of cancer by participating in competing endogenous RNA (ceRNA) networks. However, the role of survival-associated ceRNAs in breast cancer (BC) remains unknown.

Methods: The Gene Expression Omnibus database and The Cancer Genome Atlas BRCA_dataset were used to identify differentially expressed RNAs. Furthermore, circRNA-miRNA interactions were predicted based on CirInteractome, while miRNA-mRNA interactions were predicted based on TargetScan, miRDB, and miRTarBase. The survival-associated ceRNA networks were constructed based on the predicted circRNA-miRNA and miRNA-mRNA pairs. Finally, the mechanism of miRNA-mRNA pairs was determined. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of survival-related mRNAs were performed using the hypergeometric distribution formula in R software. The prognosis of hub genes was confirmed using gene set enrichment analysis.

Results: Based on the DE-circRNAs of the top 10 initial candidates, 162 DE-miRNAs and 34 DE-miRNAs associated with significant overall survival were obtained. The miRNA target genes were then identified using online tools and verified using the Cancer Genome Atlas (TCGA) database. Overall, 46 survival-associated DE-mRNAs were obtained. The results of GO and KEGG pathway enrichment analyses implied that up-regulated survival-related DE-mRNAs were mostly enriched in the "regulation of cell cycle" and "chromatin" pathways, while down-regulated survival-related DE-mRNAs were mostly enriched in "negative regulation of neurotrophin TRK receptor signaling" and "interleukin-6 receptor complex" pathways. Finally, the survival-associated circRNA-miRNA-mRNA ceRNA network was constructed using 34 miRNAs, 46 mRNAs, and 10 circRNAs. Based on the PPI network, two ceRNA axes were identified. These ceRNA axes could be considered biomarkers for BC. GSEA results revealed that the hub genes were correlated with "VANTVEER_BREAST_CANCER_POOR_PROGNOSIS", and the hub genes were verified using BC patients' tissues.

Conclusions: In this study, we constructed a circRNA-mediated ceRNA network related to BC. This network provides new insight into discovering potential biomarkers for diagnosing and treating BC.

KEYWORDS

bioinformatics, breast cancer, prognosis biomarker, GEO, ceRNAs

Introduction

Breast cancer (BC) is a common malignancy among women worldwide (1). Female BC mortality peaked in 1989 and has since fallen by 42% due to earlier diagnosis and treatment advancements (2), with 5-year survival rates for all stages reaching up to 90% (2). Despite high survival rates in women, there is still a lack of suitable molecular biomarkers for early diagnosis and targets for precision therapy.

Circular RNAs (circRNAs) are novel non-coding RNAs produced by precursor mRNA back-splicing or skipping events. They lack 5' caps and 3' poly(A) tails (3, 4) and are widely expressed across species. Owing to their high abundance, evolutionary conservation, and stability, circRNAs could be biomarkers for clinical diseases (3, 5). Several studies have shown that circRNAs correlate with carcinogenesis and patient progression (6–8); for example, circHMGCS1 could promote hepatoblastoma cell growth (9), and circANKS1B induces BC metastasis regulated by ESRP1 (10). Although few studies have reported differential expression of circRNAs, the potential biological function and underlying mechanisms of circRNAs remain unclear.

MicroRNAs (miRNAs) are highly conserved small non-coding RNAs regulate gene expression through posttranslational inhibition or target mRNA degradation (3). miRNAs could act as tumor inhibitors in various cancers, including BC (11, 12). However, the upstream mechanisms of miRNAs remain unclear. The competing endogenous RNA (ceRNA) theory, in which non-coding RNAs regulate mRNA expression by competitively binding to shared microRNA response elements (MREs), has been hypothesized (13). According to some studies, circRNAs are a new member of the ceRNA family with a key role in cancer gene expression and regulation (14, 15).

Here, we constructed a ceRNA network associated with survival in differentially expressed (DE) genes to demonstrate the molecular mechanisms underlying BC. CircRNA (GSE101123, GSE101124) and miRNA (GSE97811) expression profiles were obtained from the Gene Expression Omnibus (GEO) database. The top ten DE circRNAs were then selected as initial candidates based on their *p*-value. The prognosis-related circRNA–miRNA–mRNA network was then constructed based on circRNA–miRNA interactions and miRNA–mRNA interactions predicted using online tools. Furthermore, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed to explore the mechanism of DE mRNAs. The hub genes of mRNAs were filtered out using the Cytoscape cytoHubba plugin. Finally, an integrated circRNA–mRNA network was successfully constructed. The ceRNA network provides an exact and reliable result for further studies. A concise workflow outline of the procedure is depicted in **Figure 1**.

Materials and methods

Microarray data information and identification of the DE circRNAs, miRNAs, and mRNAs

The expression data of circRNAs, miRNAs, and mRNAs were obtained from the GEO database (<http://www.ncbi.nlm.nih.gov/geo/>), an international public and research repository platform. GSE101123 and GSE101124, based on the GPL19978 platform, are circRNA databases of BC, and both contain data from eight cancer tissue specimens and three normal tissue specimens. GSE97811 is a miRNA database that includes 45 cancer and 16 normal tissues based on the GPL21263 platform. The Cancer Genome Atlas (TCGA) TCGA_BRCA mRNA dataset, which contained 1,102 cancer tissues and 113 normal tissues, was downloaded using the TCGA-Assembler package (Version 2.0) based on the gene.normalized_RNAseq assay platform. **Table 1** provides basic information on these gene expression profiles.

The gene normalized signal intensity contained in the series matrix file and GPL probe information text was retrieved from each gene chip based on the GEO database. The circle database Hg19 was obtained from Circbase (<http://www.circbase.org/>) database. The NCBI (National Center for Biotechnology Information) BLAST (Basic Local Alignment Search Tool) Tool (Version 2.2.29) was used to get the probes to the paired official circle RNAs with an identity equal to 100 as the matched criterion. Then, the official circle RNAs normalized signal intensity matrix was standardized using the Z score standardization formula $[(x - \mu) / \sigma]$, *x*: value, μ : average, σ : standard deviation (16) performed using the Perl (Practical Extraction and Report Language, Version 5.30.2) software.

The DE circRNAs and miRNAs were identified using the limmapackage (version 3.30.0) of R (Version 3.5.1) software. DE circRNAs and miRNAs with |Fold change|(FC) > 1 and *p*-value < 0.05 were considered significant. For the DE circRNAs, we used the Venn software (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) to obtain the common DE circRNAs in two independent cohorts. The DE mRNAs were identified using the DEseq package (Version 1.42.0) of R software with the same criteria as DE circRNAs.

Prediction of circRNA–miRNA pairs

The circBase online website (<http://www.circbase.org/>) was used to obtain the basic data on circRNAs (17). The target miRNAs and miRNA response elements (MREs) present in circRNAs were predicted using The Circular RNA Interactome (CircInteractome, <https://circinteractome.nia.nih.gov/>) (18). The predicted miRNAs were further selected as candidate target miRNAs for the DE circRNAs based on DE miRNAs of GSE97811.

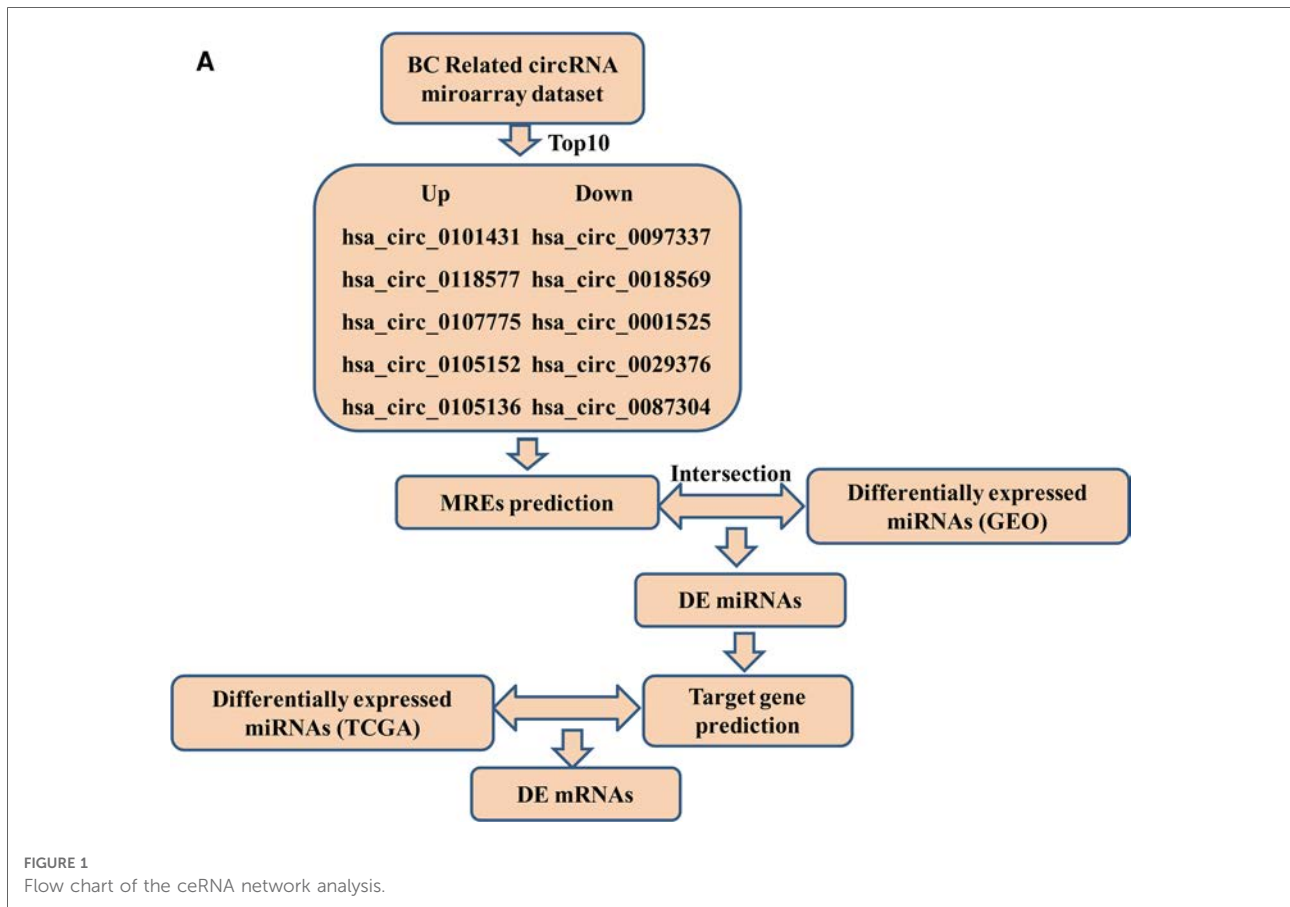


TABLE 1 Basic information from the GEO microarray and TCGA RNA-seq datasets.

Data source	Platform	Tissue	Sample size (Tumor/Normal)	Author	Year	Region	RNA type
GSE101123	GPL19978	Breast	8/3	Jianzhen Xu	2018	China	CircRNA
GSE101124	GPL19978	Breast	8/3	Jianzhen Xu	2018	China	CircRNA
GSE97811	GPL21263	Breast	45/16	AI Mitsuhashi	2017	Japan	miRNA
TCGA_BRCA	gene.normalized_RNAseq	Breast	1102/113			USA	mRNA

Survival analysis of De miRNAs

Kaplan-Meier plotter (<http://kmplot.com/analysis/>) analysis was performed to determine the survival-associated DE miRNAs. The BC database was used to estimate the prognostic values of the predicted DE miRNAs. The DE miRNA was divided into two groups based on median expression levels. $p \leq 0.05$ was considered statistically significant.

Prediction of miRNA-mRNA pairs

The miRNA-mRNA interactions were predicted using three online tools, including TargetScan (<http://www.targetscan.org/>) (19), miRDB (<http://www.mirdb.org/>) (20), and miRTarBase ([\[mirtarbase.mbc.nctu.edu.tw/php/index.php\]\(http://mirtarbase.mbc.nctu.edu.tw/php/index.php\)\) \(21\). The interactions predicted by all three websites were selected as candidate targets to intersect with the DE mRNAs from the TCGA_BRCA dataset.](http://</p>
</div>
<div data-bbox=)

Identification of prognostic predictors and construction of survival-related ceRNA networks

The survival-related DE mRNAs were selected using the same method as the DE miRNAs. Furthermore, a survival-related miRNA-miRNA-mRNA regulatory network was then constructed using a combination of circRNA-miRNA and miRNA-mRNA pairs. Cytoscape (<http://cytoscape.org/>; version 3.7.1) software was used for visualization (22). The top first

degree, obtained using the Cytoscape cytoHubba plugin, was regarded as hub genes nodes.

GO and KEGG enrichment analysis of survival-associated mRNAs

GO and KEGG annotations were downloaded from their official websites (<http://current.geneontology.org/products/pages/downloads.html>, https://www.genome.jp/kegg-bin/get_htext?hsa00001+3101,2021.01.05download). The above original file combined with DE mRNAs were tidied into a 2×2 contingency table format using the Perl software, so that the survival-related DE mRNAs could be performed using the hypergeometric distribution formula of R software. We considered a p -value ≤ 0.05 and counted >2 as a statistically significant difference and significant enrichment, respectively.

Hub gene GSEA

GSEA was performed to verify the prognosis of hub genes in BC and reveal the underlying pathway. The expression levels of genes from the genes chips were divided into two using the median. Moreover, the potential function of the hub genes was analyzed using the GSEA (version 4.1.0) (23).

Breast cancer tissues

A total of 9 BC patients treated at the Department of Breast Surgery, The Affiliated Yantai Yuhuangding Hospital of Qingdao University were selected in our study. All patients signed informed consent forms approved by the Institutional Review Board of Yantai Yuhuangding Hospital (Certificate number: 2022-245) (23, 24).

RNA isolation and Q-PCR

Total RNA was isolated using TRIzol reagent from the tissue samples of BC patients as per the manufacturer's direction (Shandong Sparkjade Biotechnology Co., Ltd.). After adding 0.5 μ g RNA, each sample was reversed to cDNA by the SPARK script II RT Plus Kit (Shandong Sparkjade Biotechnology Co., Ltd.). Q-PCR analysis was performed using the SYBR Green qPCR Mix kit (With ROX) (Shandong Sparkjade Biotechnology Co., Ltd.), following the manufacturer's instructions. Finally, the expression levels of mRNA were calculated using the $2^{-\Delta\Delta CT}$ formula.

Statistical analysis

All statistical analyses were performed using R statistical software. Wilcoxon test was used to compare the

differences between normal and tumor groups. A two-sided p -value < 0.05 indicated a statistically significant difference for all assays.

Results

Differential expression analysis

The basic information from three GEO datasets (GSE101123, GSE101123, and GSE97811) were used and are shown in **Table 1**. Each profile was analyzed using the limma package in R software. A $|FC| > 1$ and p -value < 0.05 were considered significant. Further, the DE circRNAs from the two circRNA data profiles were merged, and 249 up-regulated, and 257 down-regulated circRNAs were obtained (**Figures 2E,F**). The DE circRNAs were visualized using the heat map, volcano plot, and vein plot (**Figures 2A–D**). Simultaneously, 292 up-regulated and 1,262 down-regulated DE miRNAs were visualized using the heat map and volcano plot (**Figures 3A,B**). Furthermore, based on the initial top 10 candidates of DE circRNAs, circRNA–miRNA pair prediction was conducted using the online database CircInteractome. At last, the predicted miRNA response elements (MREs) of the circRNAs and DE miRNAs overlapped, and 108 up-regulated and 54 down-regulated target miRNAs based on the DE miRNAs were obtained using the Venn online tool (**Figures 3C,D**).

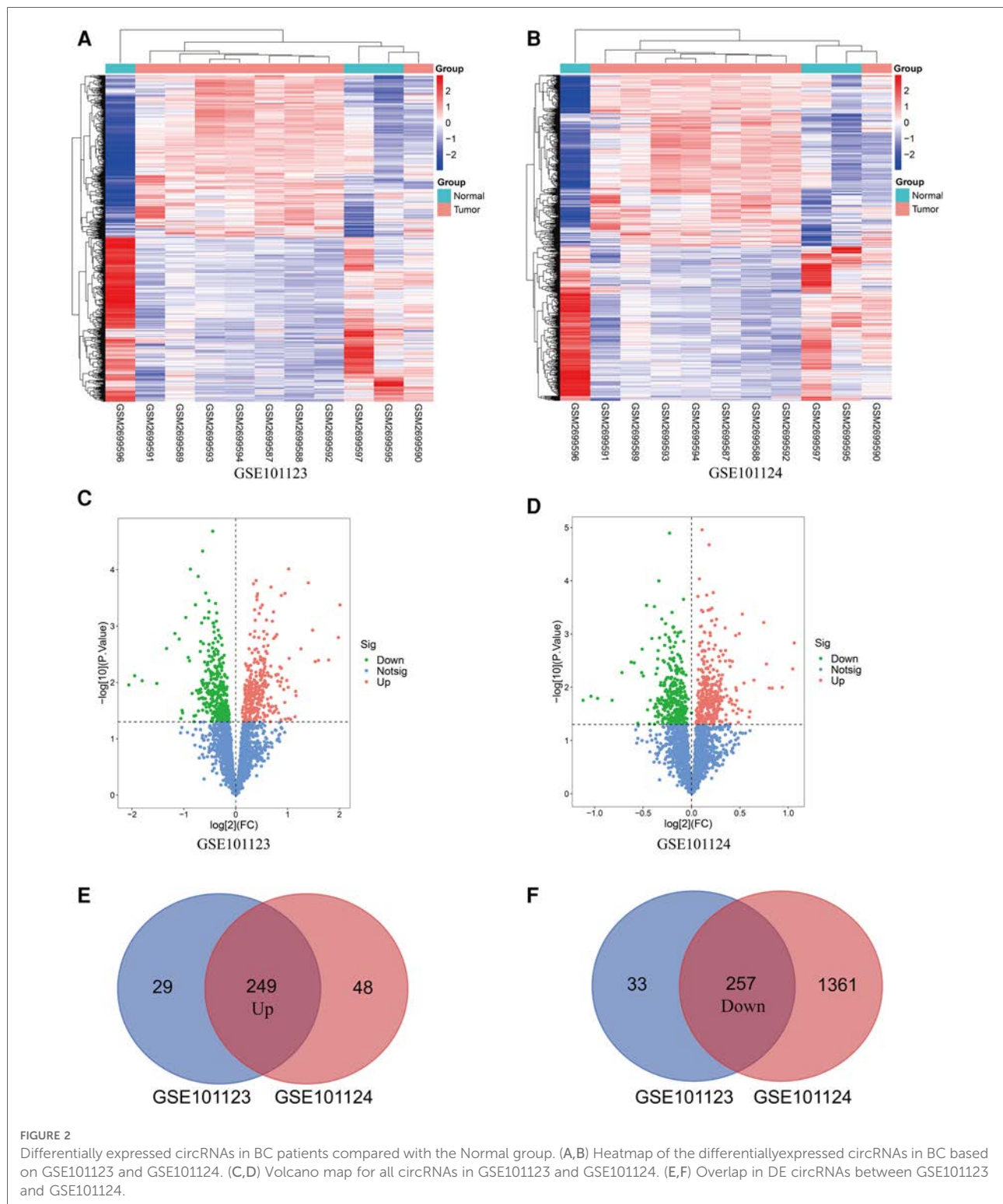
The complete TCGA_BRCA dataset of 113 normal and 1,102 cancer tissues were analyzed using the DEseq package in R software, and 3,696 up-regulated and 2,926 down-regulated DE mRNAs were identified using the same criterion as the above GEO dataset (Figure not shown).

Survival analysis of the DE miRNAs

The survival analysis of the 108 up-regulated and 54 down-regulated DE miRNAs was studied using online Kaplan-Meier Plotter tools. Finally, the 22 up-regulated and 12 down-regulated DE miRNAs confirmed our expectations. **Supplementary Figures S1, S2** depict the survival analyses of the miRNAs.

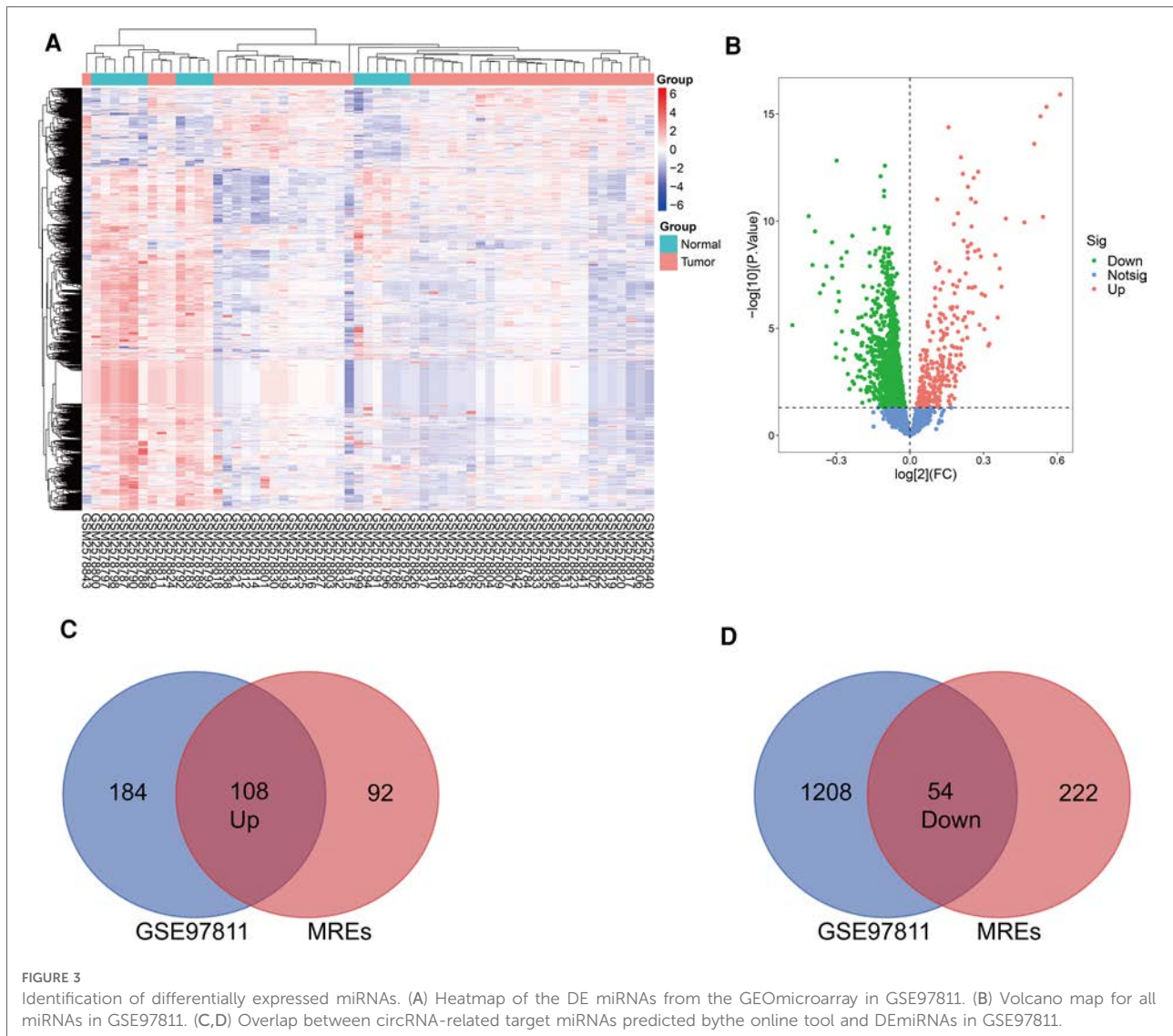
Identification of prognostic predictors and construction of survival-related ceRNA networks

TargetScan (<http://www.targetscan.org/>), miRDB (<http://www.mirdb.org/>), and miRTarBase (<http://mirtarbase.mbc.nctu.edu.tw/php/index.php>) online tools were used to predict the potential interactions between



miRNAs and mRNAs. Only target mRNAs recognized consistently through these three databases were selected as potential targets and intersected with DE mRNAs from the TCGA_BRCA dataset to identify candidate target

mRNAs for the miRNAs. Finally, using Kaplan- Meier Plotter, 18 up-regulated and 28 down-regulated survival-related mRNAs ([Supplementary Figures S3, S4](#)) were selected.

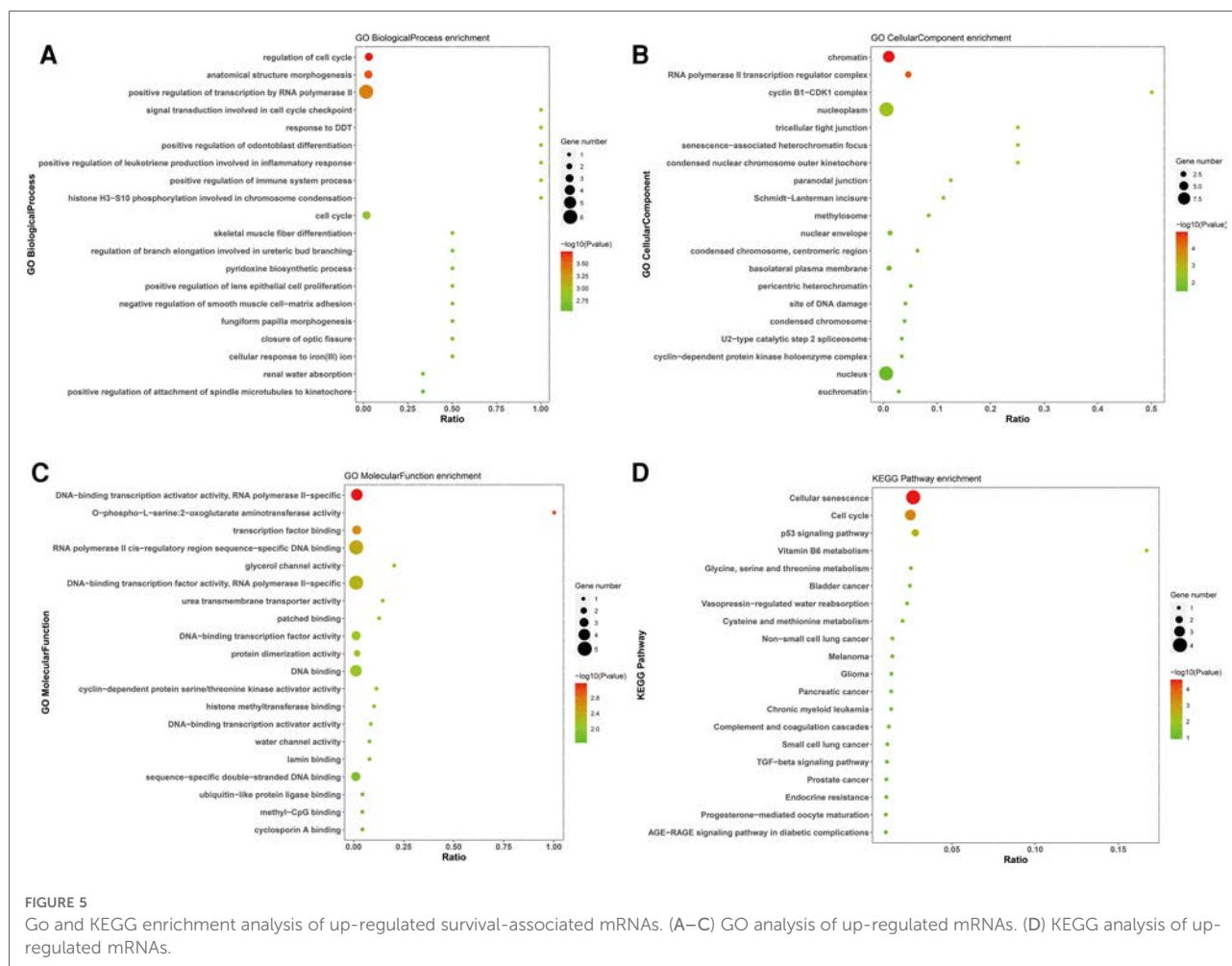


The BC survival-related circRNA-miRNA-mRNA ceRNA network was constructed using Cytoscape software (Figure 4A). The mRNAs from the ceRNA network were deposited into the STRING online database. Hub nodes of the mRNA network were identified using the Cytoscape cytoHubba plugin. Finally, two key circRNA-miRNA-mRNA axes (hsa_circ_0105136/hsa-miR-548c/CCNB1 and hsa_circ_0118577/hsa-miR-548c/CCNB1) were found.

GO and KEGG enrichment analysis of survival-associated mRNAs

GO and KEGG enrichment analyses were performed to assess the biological functions and pathways of survival-associated DE mRNAs, which consisted of three different

categories, namely, Biological Process (BP), Cell Component (CC), and Molecular Function (MF). The up-regulated survival-associated DE mRNAs were mostly involved in the “regulation of cell cycle” in the BP category, while the up-regulated survival-associated DE mRNAs were mostly involved in “chromatin” and “DNA-binding transcription activator activity, RNA polymerase II-specific” in the CC and MF categories, respectively (Figures 5A–C). The up-regulated survival-associated DE mRNAs were most abundant in the “cellular senescence” KEGG pathway (Figure 5D). The down-regulated survival-associated DE mRNAs were most abundant in the “negative regulation of neurotrophin TRK receptor signaling pathway”, “interleukin-6 receptor complex”, and “ciliary neurotrophic factor receptor activity” (Figures 6A–C). Moreover, the down-regulated survival-associated DE mRNAs were mostly associated with



regulated DE mRNAs were identified. Moreover, the DE mRNAs were consistent with the TCGA_BRCA mRNA dataset. The GO and KEGG enrichment analyses of potential DE mRNAs were carried out using the hypergeometry of R software. The up-regulated DE mRNAs were mostly involved in the “structural constituent of cytoskeleton” molecular function and the “Salmonella infection” KEGG pathway. The down-regulated DE mRNAs were mostly enriched in “signaling adaptor activity” molecular functions and “pathways in cancer” KEGG pathways. Finally, a ceRNA network was constructed. From the ceRNA network and mRNA network, we found that the *hsa_circ_0105136/hsa-miR-548c/CCNB1* and *hsa_circ_0118577/hsa-miR-548c/CCNB1* axes were the most significant. In addition, these axes were found to be correlated with the poor prognosis of BC.

Hsa_circ_0105136/hsa-miR-548c/CCNB1 is an indispensable axis in the ceRNA network, suggesting that it could be a crucial mechanism leading to BC. CCNB is highly expressed in various cancers, including colorectal cancer (28), bladder cancer (29), BC (30), and pituitary cancer (31).

Furthermore, it participates in cell proliferation, invasion, and metastasis (32). It may serve as a biomarker for estrogen receptor-positive BC (33). Simultaneously, it can activate the epithelial-mesenchymal transition (EMT) process (32). Our findings may provide new insight into the biological mechanism of CCNB1 in the development and progression of BC. Aberrant miRNAs have been reported to play an important role in the progression of cancers, including BC (32); for example, miR-125b is down-regulated in BC and inhibits cell proliferation, invasion, and migration (34, 35). miR-548 could potentially prevent HIV, HCV, and HBV infection (36). However, the role of miR-548c in the breast has not been elucidated. miR-548c belongs to the miR-548 family, and our findings revealed that it was down-regulated in BC, suggesting that high expression of miR-548c inhibits BC cell proliferation and invasion. However, the relationship between *hsa-miR-548c* and CCNB1 remains unknown. In summary, we hypothesized that *hsa_circ_0105136/hsa_circ_0118577* influenced BC progression via the miR-548c/CCNB1 axis.

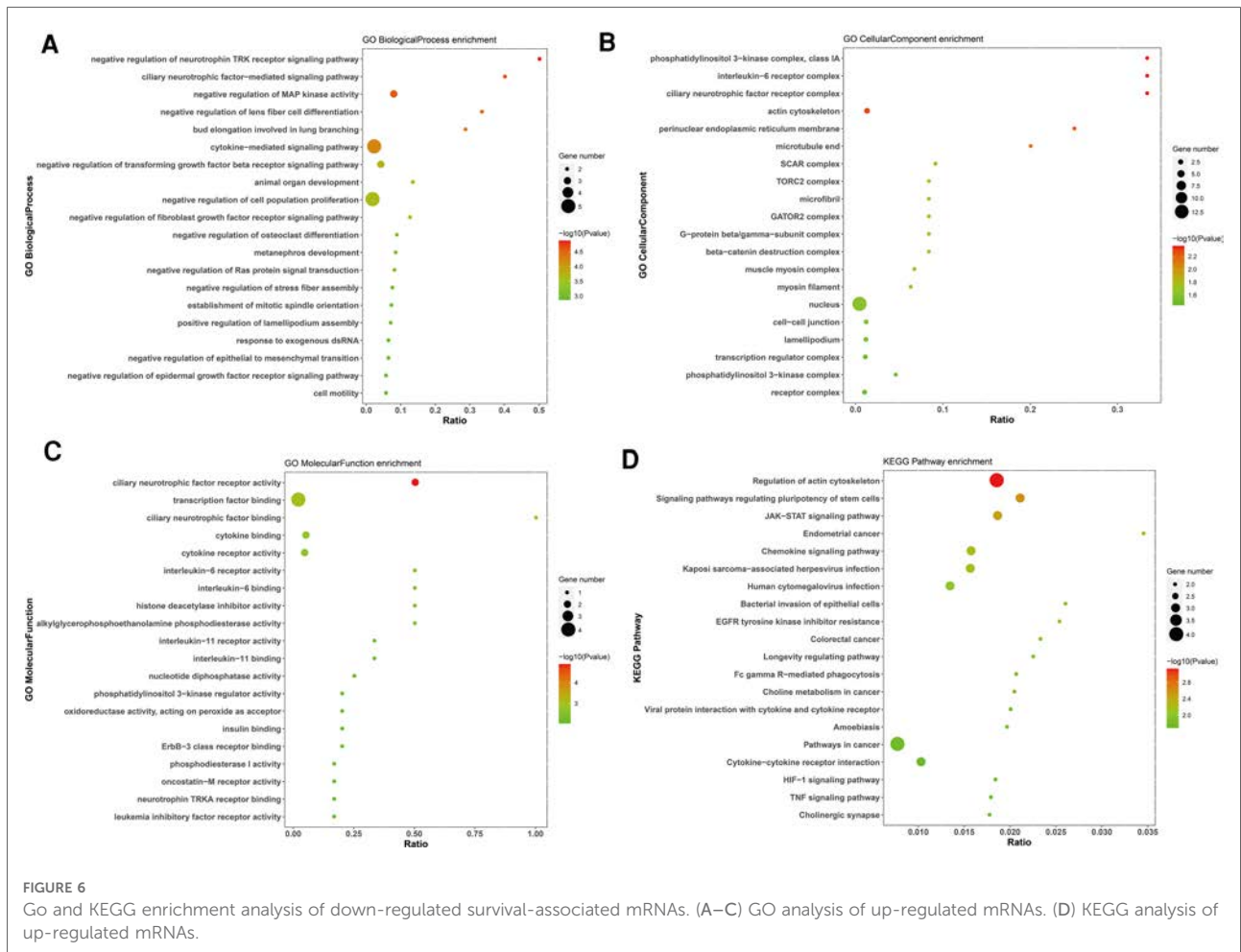


FIGURE 6 Go and KEGG enrichment analysis of down-regulated survival-associated mRNAs. (A–C) GO analysis of up-regulated mRNAs. (D) KEGG analysis of up-regulated mRNAs.

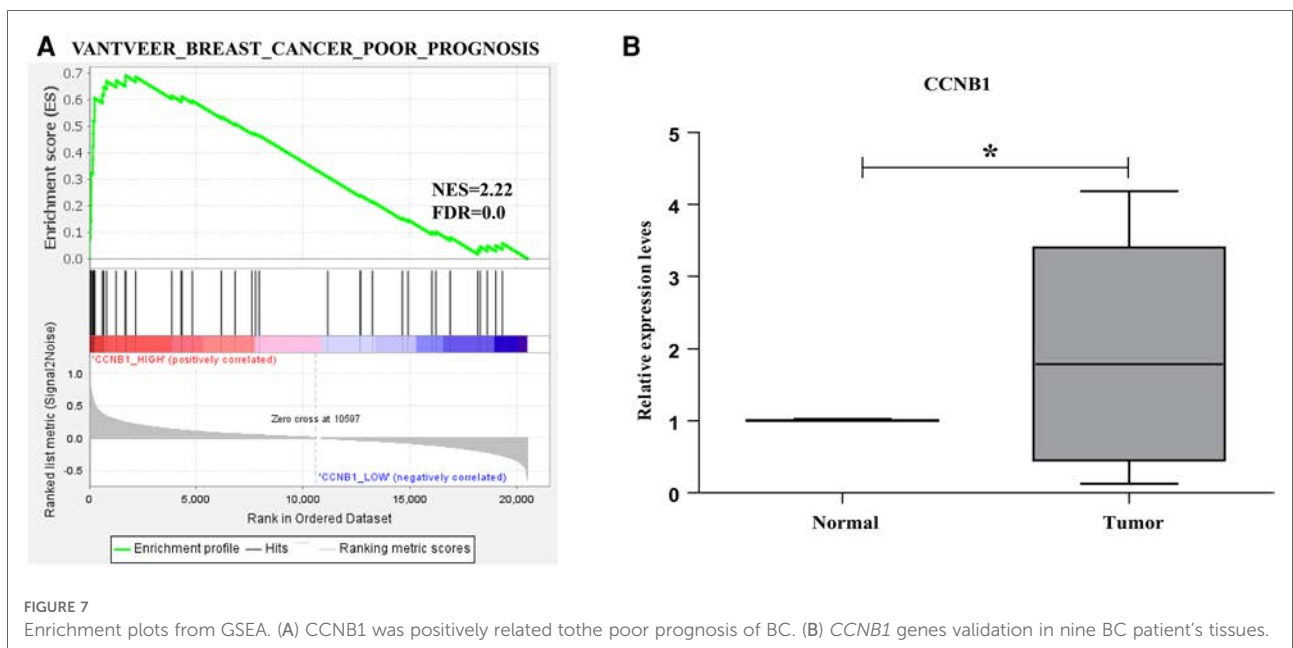


FIGURE 7 Enrichment plots from GSEA. (A) CCNB1 was positively related to the poor prognosis of BC. (B) CCNB1 genes validation in nine BC patient's tissues.

Conclusions

We constructed an underlying ceRNA network based on the above findings, which provides new insight into the potential mechanisms of BC and provides a candidate biomarker for BC. The hsa_circ_0105136/hsa-miR-548c/CCNB1 and hsa_circ_0118577/hsa-miR-548c/CCNB1 axes, in particular, may be key pathways impacting BC progression and prognosis. This analysis contributes to identifying novel diagnostic biomarkers and therapeutic targets for BC patients.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: NCBI: GSE101123, GSE97811 and GSE101124.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Board of Yantai Yuhuangding Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

GC and YL conceived, designed and wrote the manuscript; GC and JC and YD analyzed the data; GQ and YC revised the manuscript; GC and YL contributed equally to this study. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the Shandong Medical and Health Science and Technology Development Project (grant no. 202004081034) and the Science and Technology Innovation Development Plan of Yantai City (no. 2020YD013), and Special

Fund for Breast Disease Research of Shandong Medical Association (no. YXH2020ZX065).

Acknowledgments

The authors gratefully acknowledge the Gene Expression Omnibus (GEO) database and the Cancer Genome Atlas (TCGA) database, which made the data available.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsurg.2022.1021195/full#supplementary-material>.

SUPPLEMENT 1

Up-regulated DE miRNAs survival lines.

SUPPLEMENT 2

Down-regulated DE miRNAs survival lines.

SUPPLEMENT 3

Up-regulated DE mRNAs survival lines.

SUPPLEMENT 4

Down-regulated DE mRNAs survival lines.

References

- Liang Y, H Zhang, Song Q, Yang X. Metastatic heterogeneity of breast cancer: molecular mechanism and potential therapeutic targets. *Semin Cancer Biol.* (2020) 60:14–27. doi: 10.1016/j.semcancer.2019.08.012
- Siegel RL, KD Miller, Fuchs A, Jemal HE. Cancer statistics, 2022. *CA Cancer J Clin.* (2022) 72(1):7–33. doi: 10.3322/caac.21708
- Zheng X, M Huang, L Xing, R Yang, X Wang, R Jiang, et al. The circRNA circSEPT9 mediated by E2F1 and EIF4A3 facilitates the carcinogenesis and

development of triple-negative breast cancer. *Mol Cancer.* (2020) 19(1):73. doi: 10.1186/s12943-020-01183-9

4. Memczak S, M Jens, A Elefsinioti, F Torti, J Krueger, A Rybak, et al. Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature.* (2013) 495(7441):333–8. doi: 10.1038/nature11928

5. Vo JN, Cieslik M, Zhang Y, Shukla S, Xiao L, Wu YM, et al. The landscape of circular RNA in cancer. *Cell.* (2019) 176(4):869–881.e13. doi: 10.1016/j.cell.2018.12.021

6. Li X, Yang L, Chen LL. The biogenesis, functions, and challenges of circular RNAs. *Mol Cell*. (2018) 71(3):428–42. doi: 10.1016/j.molcel.2018.06.034
7. Beermann J, Piccoli MT, Viereck T, Thum J. Non-coding RNAs in development and disease: background, mechanisms, and therapeutic approaches. *Physiol Rev*. (2016) 96(4):1297–325. doi: 10.1152/physrev.00041.2015
8. Zhou Z, Sun B, Huang L, Zhao S. Roles of circular RNAs in immune regulation and autoimmune diseases. *Cell Death Dis*. (2019) 10(7):503. doi: 10.1038/s41419-019-1744-5
9. Zhen N, Gu S, Ma J, Zhu J, Yin M, Xu M, et al. CircHMGC51 promotes hepatoblastoma cell proliferation by regulating the IGF signaling pathway and glutaminolysis. *Theranostics*. (2019) 9(3):900–19. doi: 10.7150/thno.29515
10. Zeng K, He B, Yang BB, Xu T, Chen X, Xu M, et al. The pro-metastasis effect of circANKS1B in breast cancer. *Mol Cancer*. (2018) 17(1):160. doi: 10.1186/s12943-018-0914-x
11. Zhang C, Wang GX, Fu B, Zhou XC, Li Y, YY L. LncRNA CASC15 promotes migration and invasion in prostate cancer via targeting miR-200a-3p. *Eur Rev Med Pharmacol Sci*. (2020) 24(13):7215. doi: 10.26355/eurrev_202007_2186612
12. Lv C, Li F, Li X, Tian Y, Zhang Y, Sheng X, et al. MiR-31 promotes mammary stem cell expansion and breast tumorigenesis by suppressing wnt signaling antagonists. *Nat Commun*. (2017) 8(1):1036. doi: 10.1038/s41467-017-01059-5
13. Salmena L, Poliseno L, Tay Y, KatsPP, Pandolfi L. A ceRNA hypothesis: the Rosetta stone of a hidden RNA language? *Cell*. (2011) 146(3):353–8. doi: 10.1016/j.cell.2011.07.014
14. Bolha L, Ravnik-Glavac M, Glavac D. Circular RNAs: biogenesis, function, and a role as possible cancer biomarkers. *Int J Genomics*. (2017) 2017:6218353. doi: 10.1155/2017/6218353
15. Kristensen LS, Hansen TB, Venoj K, Jemtsen MT. Circular RNAs in cancer: opportunities and challenges in the field. *Oncogene*. (2018) 37(5):555–65. doi: 10.1038/onc.2017.361
16. Cheadle C, Cho-Chung YS, Becker KG, Vawter MP. Application of z-score transformation to Affymetrix data. *Appl Bioinformatics*. (2003) 2(4): 209–17. PMID: 15130792
17. Glazar P, Papavasileiou P, Rajewsky N. Circbase: a database for circular RNAs. *RNA*. (2014) 20(11):1666–70. doi: 10.1261/rna.043687.113
18. Dudekula DB, AC Panda, Grammatikakis I, De S, Abdelmohsen M, Gorospe K. Circinteractome: a web tool for exploring circular RNAs and their interacting proteins and microRNAs. *RNA Biol*. (2016) 13(1):34–42. doi: 10.1080/15476286.2015.1128065
19. Agarwal V, Bell GW, Nam DP, Bartel JW. Predicting effective microRNA target sites in mammalian mRNAs. *eLife*. (2015) 4:e05005. doi: 10.7554/eLife.05005
20. Chen Y, Wang X. miRDB: an online database for prediction of functional microRNA targets. *Nucleic Acids Res*. (2020) 48(D1):D127–31. doi: 10.1093/nar/gkz757
21. Chou CH, Shrestha S, Yang CD, Chang NW, Lin YL, Liao KW, et al. miRTarBase update 2018: a resource for experimentally validated microRNA-target interactions. *Nucleic Acids Res*. (2018) 46(D1):D296–302. doi: 10.1093/nar/gkx1067
22. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. (2003) 13(11):2498–504. doi: 10.1101/gr.1239303
23. Chen G, Yu M, Cao J, Zhao H, Dai Y, Cong Y, et al. Identification of candidate biomarkers correlated with poor prognosis of breast cancer based on bioinformatics analysis. *Bioengineered*. (2021) 12(1):5149–61. doi: 10.1080/21655979.2021.1960775
24. Zhao H, Chen L, Shan Y, Chen G, Chu Y, Dai H, et al. Hsa_circ_0038383-mediated competitive endogenous RNA network in recurrent implantation failure. *Aging*. (2021) 13(4):6076–90. doi: 10.18632/aging.202590
25. Cong Z, Diao Y, Xu Y, Li X, Jiang Z, Shao C, et al. Long non-coding RNA linc00665 promotes lung adenocarcinoma progression and functions as ceRNA to regulate AKR1B10-ERK signaling by sponging miR-98. *Cell Death Dis*. (2019) 10(2):84. doi: 10.1038/s41419-019-1361-3
26. Siegel RL, Miller A, Jemal KD. Cancer statistics, 2020. *CA Cancer J Clin*. (2020) 70(1):7–30. doi: 10.3322/caac.21590
27. Luo ZB, Lai GE, Jiang T, Cao CL, Peng T, Liu FE. A competing endogenous RNA network reveals novel lncRNA, miRNA and mRNA biomarkers with diagnostic and prognostic value for early breast cancer. *Technol Cancer Res Treat*. (2020) 19:1533033820983293. doi: 10.1177/153303382098329328
28. Fang Y, Yu H, Liang X, Xu J, Cai X. Chk1-induced CCNB1 overexpression promotes cell proliferation and tumor growth in human colorectal cancer. *Cancer Biol Ther*. (2014) 15(9):1268–79. doi: 10.4161/cbt.29691
29. Kim SK, Roh YG, Park K, Kang TH, Kim WJ, Lee JS, et al. Expression signature defined by FOXM1-CCNB1 activation predicts disease recurrence in non-muscle-invasive bladder cancer. *Clin Cancer Res*. (2014) 20(12):3233–43. doi: 10.1158/1078-0432.CCR-13-2761
30. Kalinsky K, Lim EA, Andreopoulou E, Desai AM, Jin Z, Tu Y, et al. Increased expression of tumor proliferation genes in hispanic women with early-stage breast cancer. *Cancer Invest*. (2014) 32(9):439–44. doi: 10.3109/07357907.2014.958232
31. Yu G, Wang H, Yu S, Li C, Bai J, Gui S, et al. Study on miRNAs' expression for the invasion of pituitary adenomas. *Turk Neurosurg*. (2017) 10.5137/1019-5149.JTN.20760-17.1. doi: 10.5137/1019-5149.JTN.20760-17.132
32. Li B, Cheng J, Wang H, Zhao S, Zhu H, Li C, et al. CCNB1 Affects cavernous sinus invasion in pituitary adenomas through the epithelial-mesenchymal transition. *J Transl Med*. (2019) 17(1):336. doi: 10.1186/s12967-019-2088-8
33. Ding K, Li W, Zou Z, Zou C, Wang X. CCNB1 Is a prognostic biomarker for ER+ breast cancer. *Med Hypotheses*. (2014) 83(3):359–64. doi: 10.1016/j.mehy.2014.06.013
34. Mar-Aguilar F, Luna-Aguirre CM, Moreno-Rocha JC, Araiza-Chavez J, Trevino V, Rodriguez-Padilla C, et al. Differential expression of miR-21, miR-125b and miR-191 in breast cancer tissue. *Asia Pac J Clin Oncol*. (2013) 9(1):53–9. doi: 10.1111/j.1743-7563.2012.01548.x
35. Scott GK, Goga A, Bhaumik D, Berger CE, Sullivan CC, Benz CS. Coordinate suppression of ERBB2 and ERBB3 by enforced expression of micro-RNA miR-125a or miR-125b. *J Biol Chem*. (2007) 282(2):1479–86. doi: 10.1074/jbc.M609383200
36. Khokhar A, Noorali S, Sheraz M, Mahalingham K, Pace DG. Computational analysis to predict functional role of hsa-miR-3065-3p as an antiviral therapeutic agent for treatment of triple infections: hCV, HIV-1, and HBV. *Libyan J Med*. (2012) 7:19774. doi: 10.3402/ljm.v7i0.19774