Submitted: 04 October, 2022 Accepted: 08 December, 2022 Published: 15 June, 2023 DOI:10.22514/ejgo.2023.035

ORIGINAL RESEARCH

European Journal of Gynaecological Oncology

Bioinformatics-based study of the ceRNA network and the sub-network of the Ziyin Buyang Formula in premature ovarian failure due to ovarian tumour therapy

Haixia Huang[1,2], Ruxin Wang[1,2], Guicheng Xia[2,*], Yong Tan[1,2,*]

[1]First College of Clinical Medicine, Nanjing University of Chinese Medicine, 210000 Nanjing, Jiangsu, China
[2]Department of Reproductive Medicine, Affiliated Hospital of Nanjing University of Chinese Medicine, 210000 Nanjing, Jiangsu, China

***Correspondence**
20203042@njucm.edu.cn (Guicheng Xia);
20211565@njucm.edu.cn (Yong Tan)

Abstract

Ovarian tumour therapy has been known to cause ovary ageing. The competitive endogenous RNA (ceRNA) network plays an indispensable role in various diseases. We constructed the ceRNA network to examine the role of circular RNA (circRNA) in the ovaries of older women and elucidate the intervention mechanism of the Ziyin Buyang Formula. Ovarian tissue datasets were obtained from the Gene Expression Omnibus (GEO) database and screened for differentially expressed circRNAs (DECs), differentially expressed miRNA (DEMis) and differentially expressed mRNAs (DEMs). The circBase and Cancer-Specific CircRNA Databse 2.0 (CSCD) database were used to predict the miRNA response elements (MREs) of DECs. Interacting miRNAs were obtained by intersecting MREs and DEMis. Based on the interaction between circRNA-miRNA and miRNA-mRNA, the node circRNA, miRNA and mRNA were obtained. The ceRNA network was constructed using the Cytoscape software. The targets of the Ziyin Buyang Formula were obtained from the Traditional Chinese Medicine Systems Pharmacology database and related literature. The sub-networks of the interventions of the Ziyin Buyang Formula were constructed according to the target genes and the corresponding relationships in the ceRNA network. A total of seven circRNAs, six miRNAs and 224 mRNAs were distinguished as nodes from 226 DECs, 35 DEMis, 1697 DEMs, 2320 MREs, 19 intersecting miRNAs and 765 intersecting mRNAs. Moreover, 96 chemical components, 239 targets and four target genes were identified in the ceRNA network of the Ziyin Buyang Formula. This study constructed a ceRNA network for ovary ageing due to ovarian tumour therapy, and the sub-network of the interventions of Ziyin Buyang Formula was predicted, providing a novel perspective for the effective treatment of ovary ageing due to ovarian tumour therapy.

Keywords

circRNA; ceRNA; The Ziyin Buyang Formula

1. Background

In today's society, ovarian tumours are being reported frequently [1]. Chemotherapy is one of the recommended forms of treatment for ovarian tumours. However, chemotherapy often causes ovarian damage, which leads to premature ovarian failure [2, 3].

In 2011, the competing endogenous RNA (ceRNA) hypothesis, which is based on microRNA (miRNA), was first proposed by Salmena [4]. Briefly, when the same miRNA response element (MRE) is present on an mRNA, lncRNA or circRNA, it binds to the same miRNA, thus attenuating the inhibitory effect of the miRNA on another target RNA [5–7].

Circular RNA (circRNA) is a class of newly discovered non-coding RNA (ncRNA) that comprise a special covalent loop without a 5′ cap or 3′ tail. Moreover, circRNAs are unaffected by RNA exonucleases and are highly stable [8]. circRNAs have been reported to be closely associated with ageing-related diseases like cardiovascular disease and osteoporosis [9, 10]. Recent studies have found significant differences in circRNA expression between ovarian tissues in younger and older women, which affects ovarian reserve capacity and reproductive outcomes [11].

miRNAs are short, endogenous, single-stranded, non-coding small RNA molecules of approximately 22 nucleotides. miRNAs are widely expressed in female reproductive organs such as the ovaries, uterus and fallopian tubes. Additionally, abnormal miRNA expression has been reported to affect the normal growth and development of follicles and ovarian function *via* cell proliferation and apoptosis [12, 13].

Xia *et al.* [14] divided the menstrual cycle into four classical phases. Subsequently, Tan *et al.* [15] streamlined the four phases into two phases: the postmenstrual and premenstrual phases and created the Ziyin Buyang Formula. Furthermore, Tan *et al.* [16, 17] combined the formula with reproductive endocrinology methodologies to treat age-related diseases, such as Diminished Ovarian Reserve. The Ziyin Buyang Formula works by nourishing the Yin in the late follicular phase (postmenstrual phase) and tonifying the Yang in the luteal phase (premenstrual phase). The "Ziyin" component (*Angelicae Sinensis Radix, Paeoniae Radix Alba, Rehmanniae Radix Praeparata, Cornus Officinalis Sieb. Et Zucc* and *Cuscutae Semen*) promotes follicle development by nourishing the kidney Yin while the "Buyang" component (*Dipsaci Radix, Rhizoma Dioscoreae, Morindae Officinalis Radix, Epimrdii Herba* and *Psoralea corylifolia Linn*) improves endometrial receptivity by tonifying the kidney Yang [18, 19].

This study aims to determine the molecular pathways involved in the regulation of ovarian ageing using a circRNA-mediated ceRNA network and the sub-network of the intervention of the Ziyin Buyang Formula.

2. Methods

2.1 Transcriptome dataset

Using "ovarian or oocyte and age" as keywords, the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi) was used to obtain circRNA, miRNA and mRNA gene expression profiles and platform annotation files [20].

2.2 Identification of the differentially expressed circRNA, miRNA and mRNA

The "limma package" in the R language (version 4.0.2, R Foundation for Statistical Computing, Vienna, Austria) was used to compare the differential expression of circRNAs, miRNAs and mRNAs between young and older ($\geq$35 years) ovarian tissues, and the data were standardised with a filtering criterion of corrected $p < 0.05$ to obtain differentially expressed circRNAs (DECs), differentially expressed miRNAs (DEMis) and differentially expressed mRNAs. The differential genes were visualised *via* heat maps using the "pheatmap" package. Differentially expressed rat ovarian tissue mRNAs were converted to homologous human genes by using the HomoloGene (https://www.ncbi.nlm.nih.gov/homologene) database, and the results were used as differentially expressed mRNAs (DEMs).

2.3 Predicting target genes for DEMis and MREs in DECs

The circBase database (http://circbase.org) was used to obtain the chromosome locations of DECs, and the Cancer-Specific CircRNA Databse 2.0 (CSCD) database (http://geneyun.net/CSCD2) was searched to predict MREs based on the chromosome locations. Venn diagrams were used to analyse and detect the miRNAs that overlapped with the DEMs, thereby obtaining the miRNAs of interest. The MREs were predicted using TargetScan (http://www.targetscan.org) and miRDB (http://www.mirdb.org) databases to obtain the potential target genes of the intersecting miRNAs. Finally, the potential target genes were intersected with DEMs using R to obtain the intersecting mRNAs and visualised using a Venn diagram.

2.4 Constructing ceRNA networks

The circRNA-miRNA-mRNA network was constructed based on the ceRNA theory as follows: (1) DECs, DEMis and DEMs were selected as the potential target genes of MRE and miRNA. (2) The circRNA and mRNA must have an action relationship with the same miRNA. (3) miRNA and circRNA and mRNA have a negative regulatory relationship, *i.e.*, when miRNA is up-regulated, circRNA and mRNA are down-regulated and vice versa. Finally, the circRNA-miRNA-mRNA network was constructed using Cytoscape (3.8.0).

2.5 Targets of the Ziyin Buyang Formula

The Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (https://tcmsp-e.com) was used to obtain the main active ingredients of the nourishing Yin formula (*Angelicae Sinensis Radix*, *Paeoniae Radix Alba, Rehmanniae Radix Praeparata, Cornus Officinalis Sieb. Et Zucc* and *Cuscutae Semen*) and tonifying Yang formula (*Dipsaci Radix, Rhizoma Dioscoreae, Morindae Officinalis Radix, Epimrdii Herba* and *Psoralea corylifolia Linn*). The active ingredients and their relevant targets were screened according to pharmacokinetic values (oral bioavailability $\geq$30%, drug-likeness $\geq$0.18) and supplemented by relevant literature. The screened protein targets were normalised using the Uniprot database (https://www.uniprot.org).

2.6 Obtaining target genes of the Ziyin Buyang Formula in the ceRNA network

The intersection gene between the targets of Ziyin Buyang Formula and the node mRNA in the ceRNA network were the target genes of the ceRNA network regulated by Ziyin Buyang Formula.

2.7 Constructing a regulatory network for the Ziyin Buyang Formula

The ceRNA network was used to examine miRNAs that interact with target genes and circRNAs that interact with the miRNAs. Cytoscape software was used to construct a sub-network of ceRNAs that regulate ovarian function in older patients using the Ziyin Buyang Formula.

2.8 Study Flow Chart

The expression data of circRNAs, miRNAs and mRNAs were downloaded from the Gene Expression Omnibus. Following this, DECs, DEMis and differentially expressed mRNAs were screened at $p < 0.05$. Furthermore, differentially expressed mRNAs in rat were translated into homologous human genes using the HomoloGene database. The target miRNAs of DECs were predicted using circBase, whereas the target mRNAs of intersected miRNAs were predicted using TargetScan and

miRDB. Then, the co-expression circRNAs and co-expression mRNAs were obtained to merge the target circRNAs and intersected mRNAs of DEMis with DECs and DEMs, respectively. Additionally, the co-expression circRNAs, DEMis and co-expression mRNAs were intersected. Finally, the targets of the Ziyin Buyang Formula were obtained using TCMSP. The relevant target genes of the Ziyin Buyang Formula and the node mRNAs in the ceRNA network were intersected to identify the regulatory genes of Ziyin Buyang Formula (Fig. 1).

3. Results

3.1 Transcriptome data

A single ovarian granule cell circRNA dataset (GSE97193) comprising three young and three older women and one follicular fluid miRNA dataset (GSE63737) comprising five young and four older women, and one primitive follicular mRNA dataset of rats comprising three immature rats and three aged rats were obtained from the GEO database (GSE84333). The datasets were processed using the Perl software (version 5.32.1.1 https://strawberryperl.com).

3.2 Analysis of the DECs, DEMis and mRNA

We obtained a total of 226 DECs (Fig. 2A), 35 DEMis (Fig. 2B) and 2335 differentially expressed mRNAs (Fig. 2C), which were compared with the HomoloGene database to finally obtain 1697 homologous human genes.

3.3 Intersected miRNAs and intersected mRNAs

The CSCD database revealed 206 DECs that had MRE information. A total of 2320 MREs were screened using the Perl software, and 19 intersected miRNAs were obtained by intersecting MREs and DEMis (Fig. 3A). A total of 7507 potential target genes were obtained using the TargetScan and miRDB databases. The potential target genes and DEMs were intersected, obtaining 765 intersected mRNAs (Fig. 3B).

3.4 Construction of the ceRNA network

We used the Cytoscape software to demonstrate the regulatory relationship of the circRNA-miRNA-mRNA network between older and young female ovarian tissues (Fig. 4). A total of 237 nodes, 251 edges, six node miRNAs (four down-regulated and two up-regulated miRNAs in older female ovarian tissues), seven node circRNAs (five up-regulated and two down-regulated circRNAs in older female ovarian tissue) and 224 nodal mRNAs (150 up-regulated and 74 down-regulated mRNAs in older female ovarian tissues) were presented in the constructed ceRNA network.

3.5 Targets of the Ziyin Buyang Formula

The TCMSP database was used to remove duplicates, revealing 501 chemical components of the nourishing Yin formula (125 *Angelicae Sinensis Radix*, 85 *Paeoniae Radix Alba*, 76 *Rehmanniae Radix Praeparata*, 226 *Cornus Officinalis Sieb. Et Zucc* and 29 *Cuscutae Semen*) and 396 chemical components of the tonifying Yang formula (31 *Dipsaci Radix*, 71 *Rhizoma Dioscoreae*, 174 *Morindae Officinalis Radix* and 130 *Epimrdii Herba*). Subsequently, 40 active ingredients were screened in the nourishing Yin formula based on Absorption/Distribution/Metabolism/Excretion values (two A*ngelicae Sinensis Radix*, 13 *Paeoniae Radix Alba*, two *Rehmanniae Radix Praeparata*, 20 *Cornus Officinalis Sieb. Et Zucc* and 11 C*uscutae Semen*), and 64 active ingredients were screened in the tonifying Yang formula (eight *Dipsaci Radix*, 16 *Rhizoma Dioscoreae*, 20 *Morindae Officinalis Radix* and 23 *Epimrdii Herba*). Additionally, a relevant literature review revealed three active components of *Psoralea corylifolia Linn* [21–23]. After combining all the active components and deleting the repeated values, we identified 96 active components and 239 targets.

3.6 Target genes of the Ziyin Buyang Formula in the ceRNA network

After intersecting with 224 node mRNAs in the ceRNA network, a total of four target genes were obtained from 239 target genes of the Ziyin Buyang Formula (*ECE1, CDK2, RXRA* and *BACE1*).

3.7 Construction of a sub-network for the regulation of the Ziyin Buyang Formula

Based on the four target genes of the Ziyin Buyang Formula for regulating ovarian function in older patients, we identified four miRNA nodes that were related to the target genes and four circRNA nodes related to the target miRNA. *ECE1* showed a correlation with hsa-miR-661 and hsa-miR-513a-5p. Moreover, *CDK2* and *BACE1* exhibited a correlation with hsa-miR-339-5p (Fig. 5).

4. Discussion

In this study, the ceRNA regulatory network, which centres on circRNA and miRNA, was constructed, and the molecular mechanism of the intervention of the Ziyin Buyang Formula was revealed at the transcriptome level.

Advancements in RNA sequencing and bioinformatics have led to the validation of the abundant expression of circRNAs in mammalian cells and tissues. circRNAs have also been reported to play a role in cellular senescence and cell survival [24–26]. Ovarian failure often occurs after chemotherapy for ovarian tumours [2, 3], circRNAs are abnormally expressed in aging tissues, and these circRNAs are closely related to the development of functional decline diseases [27, 28]. Cellular senescence is the cellular basis of organismal ageing, which includes cell cycle arrest, the loss of cell replication capacity and mitotic arrest [28, 29]. circRNAs are involved in the regulation of cellular senescence as follows: (1) circRNAs affect the cell cycle and promote cellular senescence through cyclin-dependent kinase 2 (CDK2). (2) circRNAs bind to senescence-associated proteins ID1 and E2F1, and stress-associated proteins HIF1α and Focal Adhesion Kinase (FAK) to promote cellular senescence [28, 30–32]. (3) circRNAs promote cell and tissue senescence by inducing apoptosis and inhibiting cell proliferation [33].

CDK2 is an important member of the cyclin-dependent

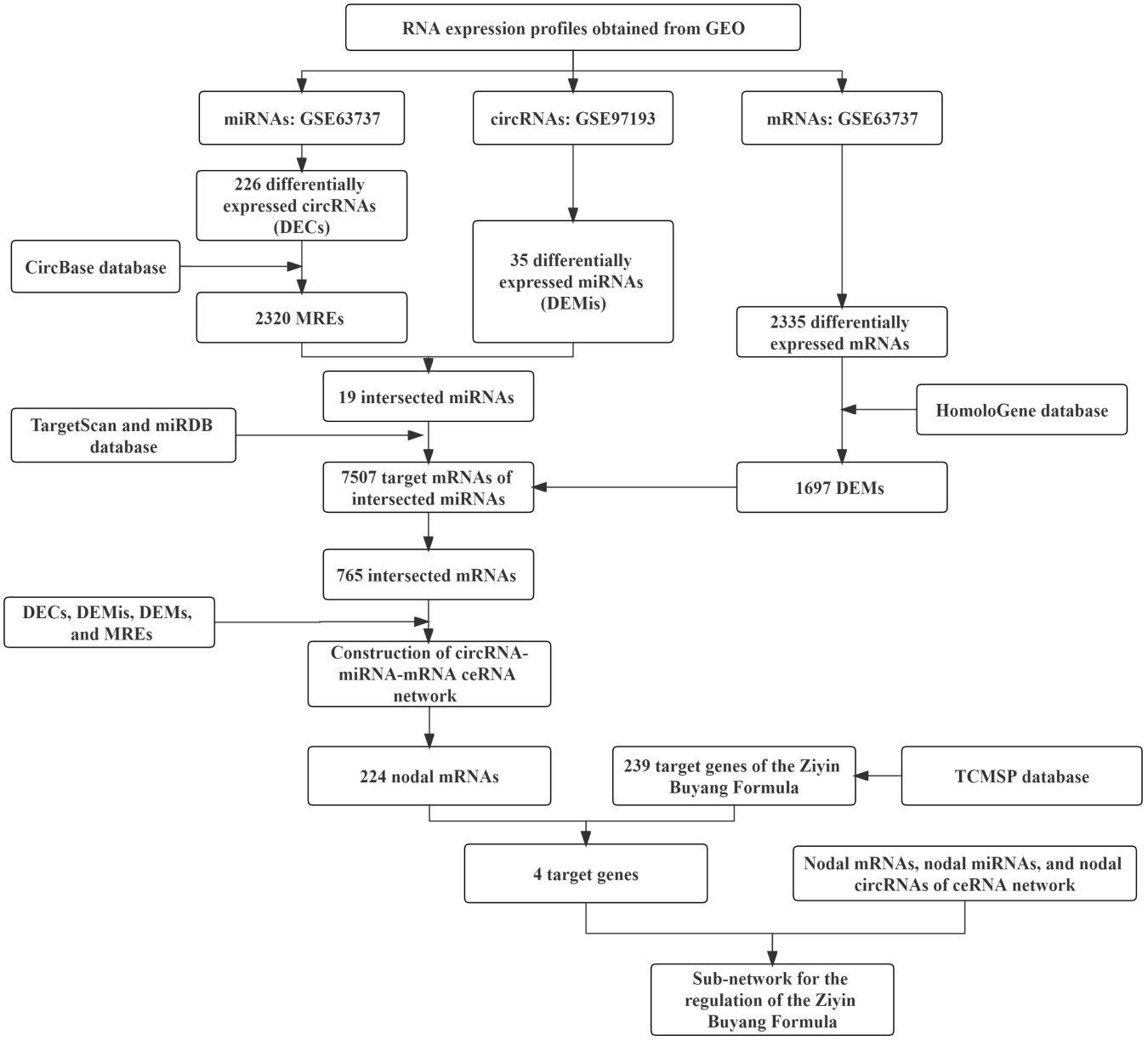

FIGURE 1. The construction of the circRNA-miRNA-mRNA network. GEO: Gene Expression Omnibus; MREs: miRNA response elements; DEMs: differentially expressed mRNAs; miRNA: microRNA; circRNA: circular RNA; TCMSP: Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; ceRNA: competitive endogenous RNA.

kinase family, which is directly involved in the G1 to S phase transition of the cell cycle. It is also a key regulator in the cell cycle, regulating DNA replication and centrosome replication during the S phase of the cell cycle [34, 35]. circRNAs regulate the ageing process and promote cell and tissue ageing by binding to CDK2 and cell cycle protein-dependent kinase inhibitor 1 [34]. Persson *et al.* [36] reported that CDK2 was abundantly expressed in mouse ovarian GCs and oocytes, which affects oocyte quality and embryo quality by dysregulating oocyte meiosis.

After searching the TCMSP database, CDK2 was identified as a potential target of *Angelicae Sinensis Radix, Cornus Officinalis Sieb. Et Zucc* and *Cuscutae Semen* in the nourishing yin formula and *Morindae Officinalis Radix, Dipsaci Radix* and *Epimrdii Herba* in the tonifying Yang formula. Combined with the ceRNA regulatory network of ovarian tissues in older patients, we hypothesised that the Ziyin Buyang Formula can delay or even reverse the process of ovarian ageing to a certain extent and improve the reproductive outcome of older patients by regulating the hsa_circ_0006520/hsa-miR-339-5p/CDK22 network.

BACE1 is an important regulator of neurodegenerative diseases and is positively correlated with the progression of the disease [37, 38]. However, a recent study on ovariectomy in rats reported that the expression level of BACE1 decreased with age after ovariectomy, which is consistent with our conclusion [39]. In this study, the expression of hsa-miR-339-5p was up-regulated while that of hsa_circ_0006520 and BACE1 were down-regulated in older ovarian tissues in the hsa_circ_0006520/hsa-miR-339-5p/BACE1 network.

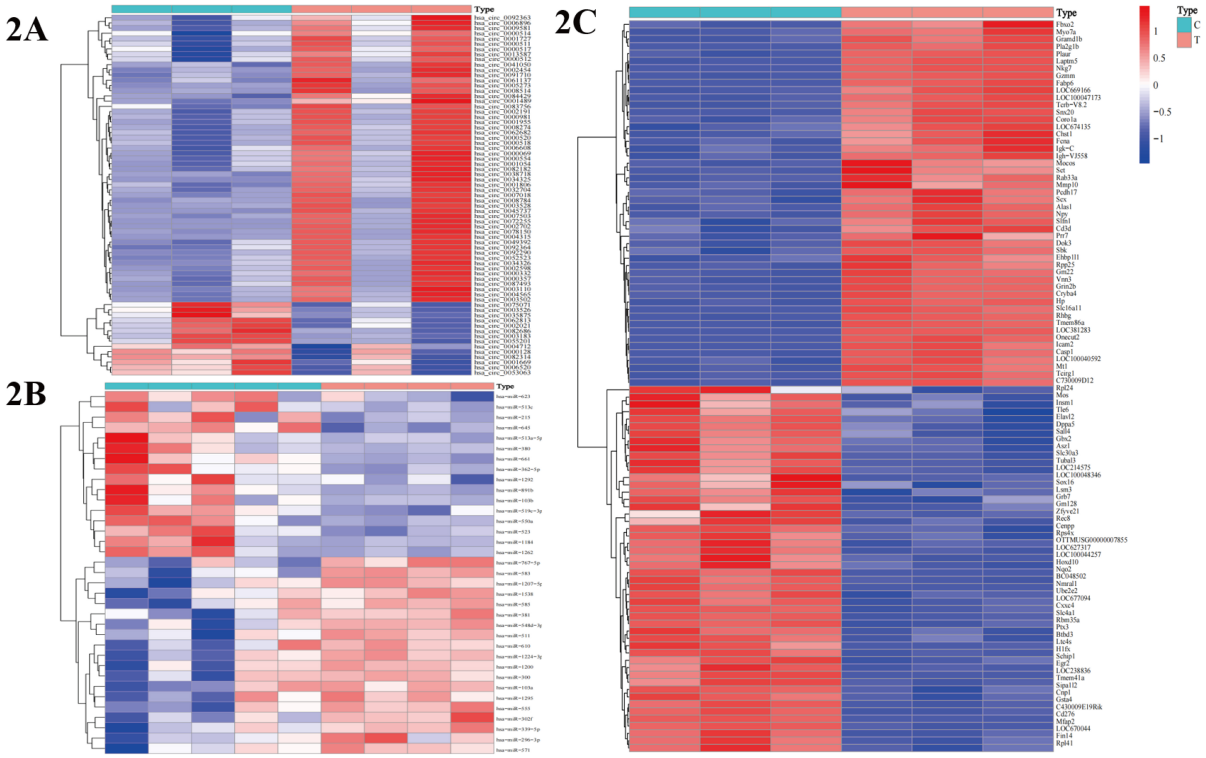

FIGURE 2. Heat map of the differentially expressed circRNA, miRNA and mRNA. Note: Pink represents older ovarian tissue, light blue represents young ovarian tissue, red indicates up-regulated gene expression and blue indicates down-regulated gene expression.

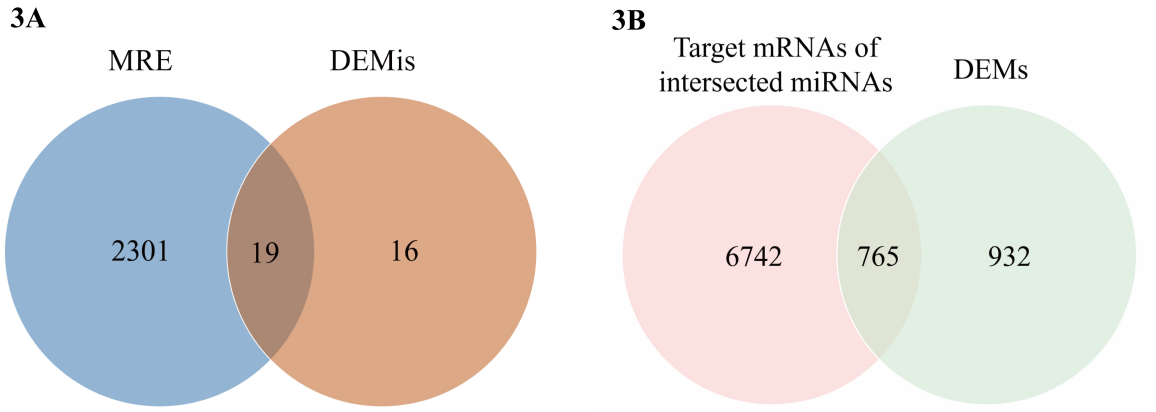

FIGURE 3. Intersected miRNAs and intersected mRNAs. Note: MRE represents the miRNA response element. DEMis represent differentially expressed miRNAs. DEMs represent differentially expressed mRNAs.

Furthermore, TCMSP identified BACE1 as a target of *Rhizoma Dioscoreae*. Moreover, some studies have demonstrated that *Rhizoma Dioscoreae* can slow down ageing through various pathways, such as antioxidant and immune enhancement. Notably, its extract, dehydroepiandrosterone, is widely used to improve assisted reproduction techniques (ART) outcomes in women with low prognoses [40–42].

Retinoid X receptor alpha (RXRA) is part of a family of nuclear receptors. Increasing evidence suggests that RXRA can regulate cellular senescence [43, 44]. Moreover, the knockdown of RXRA induced intracellular reactive oxygen species secretion and DNA damage through the ITPR2-MCU calcium signal axis, consequently activating p53 and triggering cell ageing [45]. In the hsa_circ_0082686/hsa-miR-1207-5p/RXRA network, the expression of hsa-miR-1207-5p was up-regulated with age, while that of hsa_circ_0082686 and RXRA were down-regulated, which further validated that RXRA knockdown accelerated cell ageing. Moreover, RXRA was identified as an effective target for all components in the Ziyin Buyang Formula. Thus, these findings suggest that the formula can delay ovarian ageing through the hsa_circ_0082686/ hsa-miR-1207-5p/RXRA network.

Endothelin-converting enzyme 1 (*ECE1*) induces endothelial senescence by promoting the synthesis of endothelin-1 (ET-1), which causes vascular dysfunction and promotes cell and tissue ageing [46, 47]. In the hsa_circ_0062682/hsa-

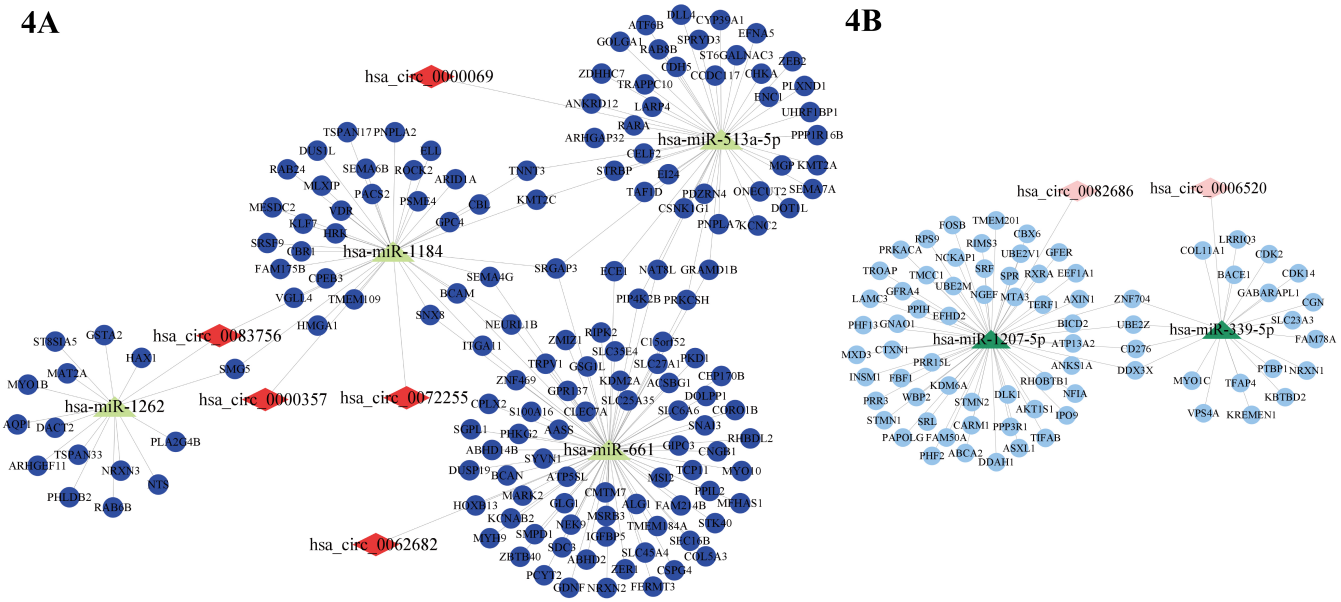

FIGURE 4. The regulatory ceRNA network of older female ovarian tissues. A, ceRNA network of circRNAs and mRNAs up-regulated in the ovaries of older patients, which is centred on miRNA down-regulation. B, ceRNA network of circRNAs and mRNAs down-regulated in the ovaries of older patients, which is centred on miRNA up-regulation. The diamonds represent circRNAs, with red and pink indicating the up-regulation and down-regulation, respectively, of circRNAa. The triangles represent miRNAs, with dark green and light green indicating the up-regulation and down-regulation, respectively of miRNAs. The circles represent mRNAs, with dark blue and light blue indicating the up-regulation and down-regulation of mRNAs.

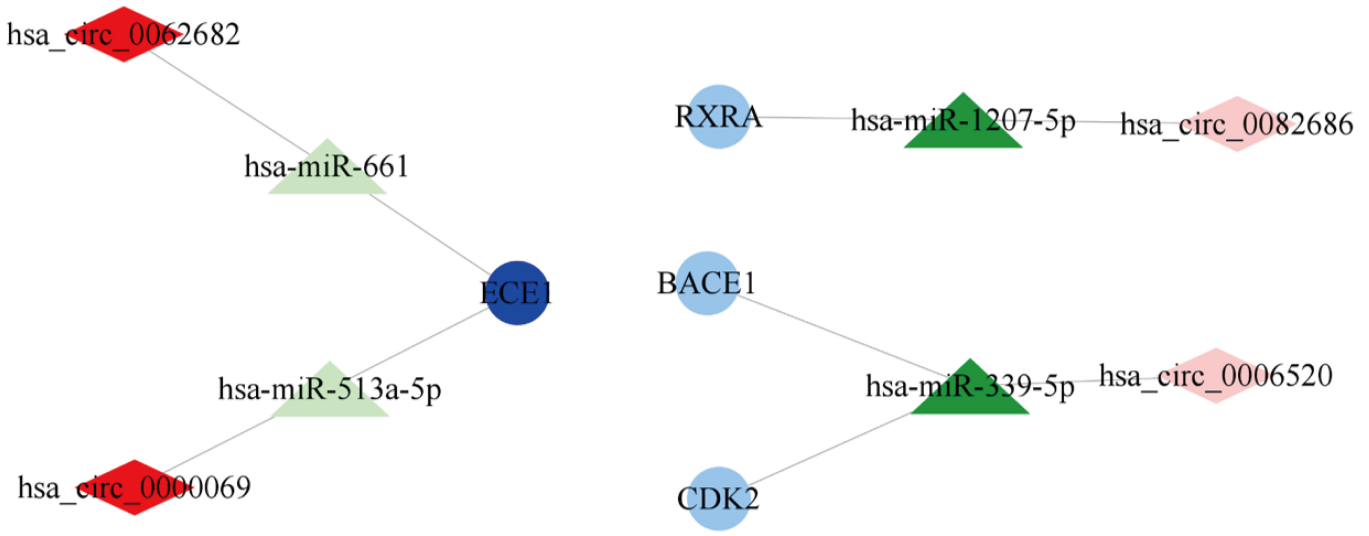

FIGURE 5. The sub-network of the Ziyin Buyang Formula for regulating ovarian function in older patients. The diamonds represent circRNAs, with red and pink indicating the up-regulation and down-regulation, respectively, of circRNAs. The triangles represent miRNAs, with dark green and light green indicating the up-regulation and down-regulation, respectively, of miRNAs. The circles represent mRNAs, with dark blue and light blue indicating the up-regulation and down-regulation, respectively, of mRNAs.

miR-661/ECE1 network, the expression of hsa-miR-661 was down-regulated and that of hsa_circ_0062682 and ECE1 were significantly up-regulated in older ovarian tissues. Furthermore, the overexpression of hsa-miR-661 induced the phosphorylation of the Phosphatidylinositol 3 kinase/Protein kinase B (PI3K/AKT) pathway, thereby promoting cell proliferation and delaying cell senescence, whereas the overexpression of ECE1 promoted cell senescence. Both hsa-miR-661 and ECE1 were reported to be expressed in ovarian tissues [46, 48, 49]. Additionally, Xie *et al.* [50] detected hsa-miR-513a-5p in ovarian tissues and found it to be closely related to the response of ovulation induction. Furthermore, in this study, ECE1 was identified as the target of *Cuscutae Semen*. Thus, combined with the ceRNA regulatory network, we determined two sub-networks of the Ziyin Buyang Formula for the regulation of ovarian function in ovarian ageing due to ovarian

tumour therapy: hsa_circ_0062682/hsa-miR-661/ECE1 and hsa_circ_0000069/hsa-miR-513a-5p/ECE1.

5. Conclusions

This study constructed a ceRNA network for patients with premature ovarian failure, revealing the specific molecular mechanisms of age-affected ovarian function and providing a new therapeutic target for improving ovarian function of patients with premature ovarian failure. Furthermore, we identified and preliminarily validated the underlying molecular mechanisms of the Ziyin Buyang Formula, thereby improving ovarian function of ovary ageing due to ovarian tumour therapy.

ABBREVIATIONS

ceRNA: competitive endogenous RNA; circRNA: circular RNA; miRNA: microRNA; ncRNA: noncoding RNA; lncRNA: long noncoding RNA; GEO: Gene Expression Omnibus; DECs: differentially expressed circRNAs; DEMis: differentially expressed microRNAs; DEMs: differentially expressed mRNAs; MRE: microRNA response element; TCMSP: Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; ART: assisted reproduction techniques; IVF-ET: *in vitro* fertilization-embryo transfer; GC: granulosa cell.

AVAILABILITY OF DATA AND MATERIALS

All data used in this study can be obtained from the mentioned open platform websites or the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

HXH and RXW—designed the research scheme, searched databases, analysed the data and drafted the manuscript. YT—obtained funding for the study, conceived and participated in its design and coordination. GCX—provided guidance and supervision.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

ACKNOWLEDGMENT

We thank Bullet Edits Limited for the linguistic editing and proofreading of the manuscript.

FUNDING

This study was supported by the National Natural Science Foundation of China (81873333), (8217151456); Jiangsu Province Graduate Student Practice innovation program (KYCX22_1931); Jiangsu Provincial Health Planning Commission Science, Education, and Health Strengthening Project and Jiangsu Provincial Clinical Medical Center of Gynecology of Traditional Chinese Medicine (YXZXB2016006).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, Gaudet MM, Jemal A, Siegel RL. Ovarian cancer statistics, 2018. CA Cancer J Clin. 2018; 68: 284-296.

[2] Jang H, Hong K, Choi Y. Melatonin and Fertoprotective Adjuvants: Prevention against Premature Ovarian Failure during Chemotherapy. Int J Mol Sci. 2017; 18: 1221.

[3] Ebrahimi M, Akbari Asbagh F. Pathogenesis and causes of premature ovarian failure: an update. Int J Fertil Steril. 2011 ;5: 54-65.

[4] Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi P. A ceRNA hypothesis: the Rosetta stone of a hidden RNA language? Cell. 2011; 146: 353–358.

[5] Boya Z, Yuxi G, Yefan Y, Xiang Z, Zhihong Z. Regulation of long noncoding Rnas *via* ceRNA in breast cancer. Chinese Journal of Clinical and Experimental Pathology. 2021; 37: 309–312. (In Chinese)

[6] Yongkang S, Zhijian Z, Xiufen Z, Bo M, Caixi T. Research progress of LncRNA MALAT1 as competitive endogenous RNA in liver cancer. Chinese Journal of Clinical and Experimental Pathology. 2021; 37: 438–440. (In Chinese)

[7] Patop I L, Wust S, Kadener S. Past, present, and future of circRNAs. The EMBO Journal. 2019; 38: e100836.

[8] Lasda E, Parker R. Circular RNAs: diversity of form and function. RNA. 2014; 20: 1829–1842.

[9] Haque S, Ames RM, Moore K, Pilling LC, Peters LL, Bandinelli S, *et al*. CircRNAs expressed in human peripheral blood are associated with human aging phenotypes, cellular senescence and mouse lifespan. GeroScience. 2020; 42: 183–199.

[10] Knupp D, Miura P. CircRNA accumulation: a new hallmark of aging? Mechanisms of Ageing and Development. 2018; 173: 71–79.

[11] Cheng J, Huang J, Yuan S, Zhou S, Yan W, Shen W, *et al*. Circular RNA expression profiling of human granulosa cells during maternal aging reveals novel transcripts associated with assisted reproductive technology outcomes. PLoS One. 2017; 12: e177888.

[12] Bahmyari S, Jamali Z, Khatami S H, Vakili O, Roozitalab M, Savardashtaki A, *et al*. microRNAs in female infertility: an overview. Cell Biochemistry and Function. 2021; 39: 955–969.

[13] Zhang J, Xu Y, Liu H, Pan Z. MicroRNAs in ovarian follicular atresia and granulosa cell apoptosis. Reproductive Biology and Endocrinology. 2019; 17: 9.

[14] Guicheng X. Regulating Menstrual Cycle with Menstrual Cycle Rhythm Induction Method. Journal of Nanjing University of Chinese Medicine (Natural Science Edition). 2000; 11–12. (In Chinese)

[15] Yong T. TAN Yong's Experience of Effective Prescriptions—Sequential Ziyin Buyang Prescription. Jiangsu Journal of Traditional Chinese Medicine. 2022; 54: 1–2. (In Chinese)

[16] Qianqian L, Guicheng X, Yong T. Fertility reduction stratagies in elderly women. China Journal of Traditional Chinese Medicine and Pharmacy. 2021; 36: 5926–5929. (In Chinese)

[17] Yuying S, Shuping C, Yong T. Effect of Nourishing Yin and Tonifying Yang Sequential therapy combined with western medicine on expression levels of Smad2/Smad3/Smad7 mRNA in rats with diminished ovarian reserve. Acta Medicinae Universitatis Scientiae et Technologiae Huazhong. 2018; 47: 300–304.

[18] Ruxin W, Yong T, Yiqing H. Analysis of experience of Tan Yong in treating diminished ovarian reserve. Clinical Journal of Traditional Chinese Medicine. 2021; 33: 1060–1064. (In Chinese)

[19] Haixia H, Yong T. A probe into Tan Yong's experience of treating infertility due to premature ovarian dysfunction. Jiangsu Journal of

Traditional Chinese Medicine. 2019; 51: 17–19. (In Chinese)
[20] Barrett T, Wilhite S E, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, *et al*. NCBI GEO: archive for functional genomics data sets—update. Nucleic Acids Research. 2013; 41: D991–D995.
[21] Hongwei L, Yangang C, Lianqi T. Five didfferent processing methods induced variations in composition and content of liposoluble and volatile constituents of Psoralea corylifolia. Chinese Traditional Patent Medicine. 2021; 43: 2418–2427. (In Chinese)
[22] Zifei Q, Peile W, Han X, Han L, Hua B, Jing Y, *et al*. Effect of prenylated conpounds derived from psoralea corylifolia against human CYP1A1 and corresponding molecular docking validation. Journal of Nanjing University of Traditional Chinese Medicine. 2021; 37: 750–759.
[23] Yali W, Xiaoyan W, Hui Z, Chen YL, Zhang ML, Chen XF, *et al*. Study on alleviating effect of Glycyrrhizae Radix et Rhizoma on Psoraleae Fructus-induced liver injury based on network pharmacology and cell experiments. China Journal of Chinese Materia Medica. 2022; 47: 176–187. (In Chinese)
[24] Chen L. The expanding regulatory mechanisms and cellular functions of circular RNAs. Nature Reviews Molecular Cell Biology. 2020; 21: 475–490.
[25] Chen L. The biogenesis and emerging roles of circular RNAs. Nature Reviews Molecular Cell Biology. 2016; 17: 205–211.
[26] Maiese K. Disease onset and aging in the world of circular RNAs. Journal of Translational Science. 2016; 2: 327–329.
[27] Fanciulli A, Wenning GK. Multiple-system atrophy. New England Journal of Medicine. 2015; 372: 249–263.
[28] Du WW, Yang W, Chen Y, Wu ZK, Foster FS, Yang Z, *et al*. Foxo3 circular RNA promotes cardiac senescence by modulating multiple factors associated with stress and senescence responses. European Heart Journal. 2017; 38: 1402–1412.
[29] Rodier F, Campisi J. Four faces of cellular senescence. Journal of Cell Biology. 2011; 192: 547–556.
[30] Perk J, Iavarone A, Benezra R. Id family of helix-loop-helix proteins in cancer. Nature Reviews Cancer. 2005; 5: 603–614.
[31] Iglesias-Ara A, Zenarruzabeitia O, Fernandez-Rueda J, Sánchez-Tilló E, Field SJ, Celada A, *et al*. Accelerated DNA replication in E2F1- and E2F2-deficient macrophages leads to induction of the DNA damage response and p21CIP1-dependent senescence. Oncogene. 2010; 29: 5579–5590.
[32] Nowicki TS, Zhao H, Darzynkiewicz Z, Moscatello A, Shin E, Schantz S, *et al*. Downregulation of uPAR inhibits migration, invasion, proliferation, FAK/PI3K/Akt signaling and induces senescence in papillary thyroid carcinoma cells. Cell Cycle. 2011; 10: 100–107.
[33] Holdt LM, Stahringer A, Sass K, Pichler G, Kulak NA, Wilfert W, *et al*. Circular non-coding RNA ANRIL modulates ribosomal RNA maturation and atherosclerosis in humans. Nature Communications. 2016; 7: 12429.
[34] Du WW, Yang W, Liu E, Yang Z, Dhaliwal P, Yang BB. Foxo3 circular RNA retards cell cycle progression *via* forming ternary complexes with p21 and CDK2. Nucleic Acids Research. 2016; 44: 2846–2858.
[35] Sandhu C, Donovan J, Bhattacharya N, Stampfer M, Worland P, Slingerland J. Reduction of Cdc25a contributes to cyclin E1-Cdk2 inhibition at senescence in human mammary epithelial cells. Oncogene. 2000; 19: 5314–5323.
[36] Persson JL, Zhang Q, Wang XY, Ravnik SE, Muhlrad S, Wolgemuth DJ. Distinct roles for the mammalian A-type cyclins during oogenesis. Reproduction. 2005; 130: 411–422.
[37] Wang R, Wu Z, Liu R, Bai L, Lin Y, Ba Y, *et al*. Age-related miRNAs dysregulation and abnormal BACE1 expression following Pb exposure in adolescent mice. Environmental Toxicology. 2022; 37: 1902–1913.
[38] Yan R, Fan Q, Zhou J, Vassar R. Inhibiting BACE1 to reverse synaptic dysfunctions in Alzheimer's disease. Neuroscience & Biobehavioral Reviews. 2016; 65: 326–340.
[39] Agca C, Klakotskaia D, Stopa EG, Schachtman TR, Agca Y. Ovariectomy influences cognition and markers of Alzheimer's disease. Journal of Alzheimer'S Disease. 2020; 73: 529–541.
[40] Cong H, Xianglong M, Chenxu N, Xiaojuan S, Shuosheng Z. Research progress of Chinese Yam and its network pharmacological analysis of anti-aging. Modernization of Traditional Chinese Medicine and Materia Medica—World Science and Technology. 2020; 22: 2348–2365. (In Chinese)
[41] Piette PCM. Questionable recommendation for LPS for IVF/ICSI in ESHRE guideline 2019: ovarian stimulation for IVF/ICSI. Human Reproduction Open. 2021; 2021: hoab005.
[42] Ovarian ST, Bosch E, Broer S, Griesinger G, Grynberg M, Humaidan P, *et al*. ESHRE guideline: ovarian stimulation for IVF/IC†. Human Reproduction Open. 2020; 2020: hoaa009.
[43] Graziano S, Johnston R, Deng O, Zhang J, Gonzalo S. Vitamin D/vitamin D receptor axis regulates DNA repair during oncogene-induced senescence. Oncogene. 2016; 35: 5362–5376.
[44] Sever R, Glass CK. Signaling by nuclear receptors. Cold Spring Harbor Perspectives in Biology. 2013; 5: a016709–a016709.
[45] Ma X, Warnier M, Raynard C, Ferrand M, Kirsh O, Defossez P, *et al*. The nuclear receptor RXRA controls cellular senescence by regulating calcium signaling. Aging Cell. 2018; 17: e12831.
[46] Olmos G, Martínez-Miguel P, Alcalde-Estevez E, Medrano D, Sosa P, Rodríguez-Mañas L, *et al*. Hyperphosphatemia induces senescence in human endothelial cells by increasing endothelin-1 production. Aging Cell. 2017; 16: 1300–1312.
[47] Hansen KE, Johnson MG, Carter TC, Mayer J, Keuler NS, Blank RD. The −839 (A/C) polymorphism in the *ECE1* isoform b promoter associates with osteoporosis and fractures. Journal of the Endocrine Society. 2019; 3: 2041–2050.
[48] Zhu T, Yuan J, Wang Y, Gong C, Xie Y, Li H. MiR-661 contributed to cell proliferation of human ovarian cancer cells by repressing INPP5J expression. Biomedicine & Pharmacotherapy. 2015; 75: 123–128.
[49] Yoshioka S. Endothelin-converting enzyme-1 is expressed on human ovarian follicles and corpora lutea of menstrual cycle and early pregnancy. Journal of Clinical Endocrinology & Metabolism. 1998; 83: 3943–3950.
[50] Xie S, Batnasan E, Zhang Q, Li Y. MicroRNA expression is altered in granulosa cells of ovarian hyperresponders. Reproductive Sciences. 2016; 23: 1001–1010.

How to cite this article: Haixia Huang, Ruxin Wang, Guicheng Xia, Yong Tan. Bioinformatics-based study of the ceRNA network and the sub-network of the Ziyin Buyang Formula in premature ovarian failure due to ovarian tumour therapy. European Journal of Gynaecological Oncology. 2023; 44(3): 35-42. doi: 10.22514/ejgo.2023.035.