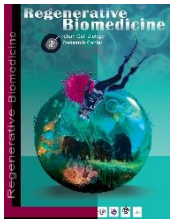

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Original Article

Multi-Tissue ceRNA Network Elucidates Exosome-Mediated Pathogenesis of Premature Ovarian Insufficiency with Implications for the Stem Cell Niche

Seyedeh Mahdieh Moghimi-Moghadam[1,2], Amirreza Cheraghinik[1], Zahra Ahmadnia[1], Seyed Mehdi Hoseini[3,4], Fateme Montazeri[1*]

[1] Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
[2] Department of Biology, Yazd University, Yazd, Iran.
[3] Hematology and Oncology Research Center, Non-communicable Diseases Research Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
[4] Biotechnology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

***Corresponding Author:**
Montazeri, Fateme
Email:
marjan.montazeri@gmail.com

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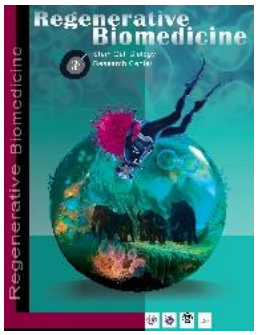
Abstract

Premature Ovarian Insufficiency (POI) remains a significant cause of female infertility, yet its molecular mechanisms are incompletely understood. This study employed an integrative bioinformatics approach using multiple GEO datasets to construct a comprehensive ceRNA network across granulosa cells, cumulus cells, and exosomal miRNAs in POI. We identified distinct molecular signatures: granulosa cells showed cell cycle disruption, while cumulus cells exhibited differentiation pathway dysregulation. The core ceRNA network revealed 4 lncRNAs potentially regulating 31 mRNAs through sponging 4 key miRNAs (miR-423-5p, miR-106b-5p, miR-452-5p, and miR-3613-5p). Functional enrichment of these mRNAs implicated key stem cell-related pathways, including pluripotency and Hippo signaling. These findings provide novel insights into POI pathogenesis through exosome-mediated regulatory mechanisms, suggesting potential therapeutic targets for ovarian dysfunction and highlighting specific implications for the ovarian stem cell niche.

Keywords: Bioinformatics Analysis, ceRNA Network, Exosomal miRNAs, Premature Ovarian Insufficiency (POI), Stem Cell Niche



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Introduction

Premature ovarian insufficiency (POI) is defined as the loss of ovarian function before age 40, affecting some women of childbearing age(1). POI is highly heterogeneous in genetic etiology. Yet identifying causative genes has been challenging with candidate gene approaches(2). In clinics, POI is divided into three stages, namely the insidious, biochemical, and evident (previously known as premature ovarian failure). Regular menstrual cycles are observed in patients with biochemical premature ovarian failure (bPOI), but they also have high FSH levels and reduced fertility(3). The available studies suggest that POIs are multi-layered and sophisticated pathogenic processes involving genetic, autoimmune, metabolic disorders, and infections. that POIs are multi-layered and sophisticated pathogenic processes involving genetic, autoimmune, metabolic disorders, and infections. To date, the pathogenesis of POI is not clear. Some researchers found that apoptosis of granulosa cells is present in all cases of premature ovarian failure. Granulosa cells (GCs), essential follicle somatic components are crucial to the process of folliculogenesis(4). Recent studies suggest that dysfunction of GCs in patients with POI may contribute to the development of ovarian insufficiency(5). Cumulus cells, a subset of granulosa cells, maintain close contact with the oocyte, providing a variety of nourishing and metabolic factors. Both cumulus and granulosa cells are primary sources of estradiol. High levels of estradiol prevent the rise of Follicle-Stimulating Hormone (FSH), a necessary factor for follicular growth and ovulation induction, thereby affecting ovulation(6, 7). Studies comparing gene expression arrays in ovarian tissue, granulosa cells, and oocytes of POI patients and controls have reported similar pathways of differentially expressed genes (DEGs). With a more profound understanding of genomics, long non-coding RNAs (lncRNAs) and microRNAs (miRNAs), these non-coding RNAs (ncRNAs) of the human transcriptome have received increasing attention from researchers. Numerous studies have confirmed that microRNAs serve as pragmatic markers for diagnosis and play an essential role in the pathogenesis of various diseases. Besides, ncRNAs are involved in multiple complex networks of many molecular mechanisms that regulate genomic function and competitive RNA-RNA interactions. Competitive endogenous RNAs (ceRNAs) are a recently discovered mechanism involving complex gene regulation between ncRNAs and mRNAs.(8) Competing for shared miRNAs allows transcripts in the ceRNA network to control one another at the post-transcriptional level, indicating that the interaction between lncRNA, miRNA, and mRNA has a significant impact on many diseases.Recent studies have constructed ceRNA networks in various diseases that help reveal the pathogenesis of diseases and provide potential therapeutic sites for disease treatment. by analyzing the expression data of granulosa cells, cumulus cells, and exosomal miRs in POI patients in the Gene Expression Omnibus (GEO) database, we constructed a lncRNA-miRNA-mRNA network in the present study to further reveal the interaction of ceRNAs underlying POI and provide new insights for future studies on the pathological basis of

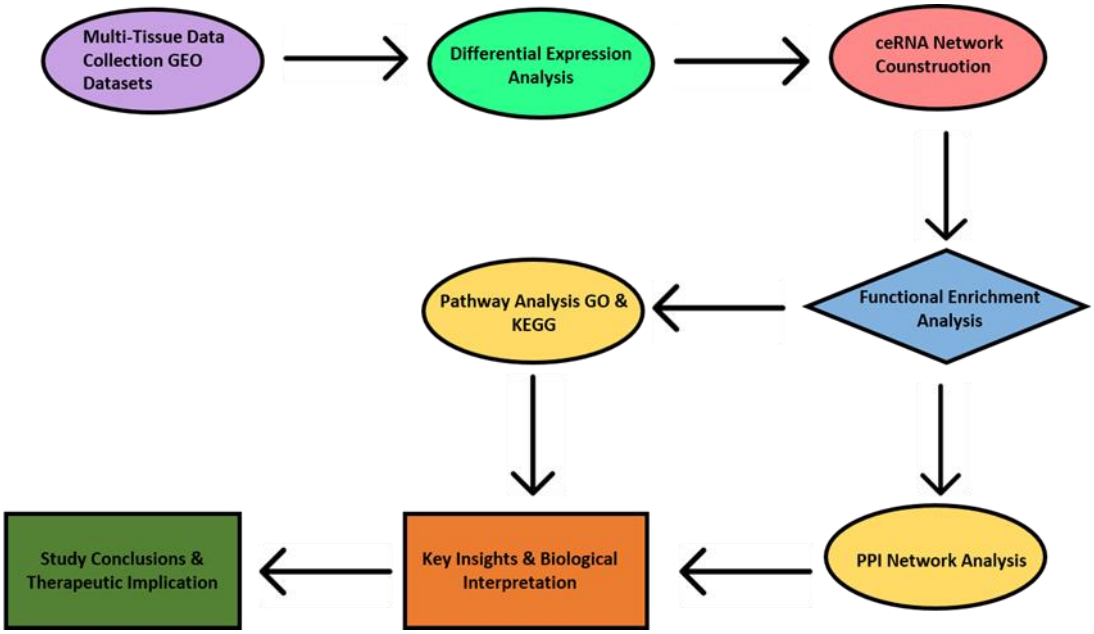


Figure 1. Workflow of Integrated Multi-tissue ceRNA Analysis in POI:This schematic illustrates the comprehensive bioinformatics pipeline, from multi-tissue data integration and differential expression analysis to ceRNA network construction and functional validation, revealing exosome-mediated regulatory mechanisms in POI with therapeutic implications.

POI(Fig. 1). We also identified chemicals that have the ability to react with important POI genes to adopt a suitable strategy for the treatment of POI.

Materials and methods

Data collection

To identify transcriptomic and epigenomic datasets related to premature ovarian insufficiency (POI), a systematic search was conducted in the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/).The search was performed using the keywords *"premature ovarian insufficiency"*, *"premature ovarian failure"*, *"POI"*, *"granulosa cells"*, *"cumulus cells"*, *"exosomal miRNA"*, and *"human"*. After screening the search results, four datasets that met the inclusion criteria were selected for . In this study, four publicly available GEO datasets related to premature ovarian insufficiency (POI) were included for downstream bioinformatics analyses: GSE201276, GSE267641, GSE253684, and GSE158526. GSE201276 comprises mRNA expression data obtained by RNA-seq of granulosa cells from 6 POI patients and 5 matched controls. The sequencing was done on the HiSeq X Ten platform (GPL20795) (4). GSE267641 contains methylation and hydroxymethylation profiles at single-base resolution in cumulus cells of POI versus control groups, enabling integrative epigenetic analysis (9). GSE253684 captures exosomal miRNA expression in follicular fluid, comparing POI patients to controls. The data were generated using Illumina HiSeq 2500. GSE158526 includes lncRNA and mRNA expression profiles generated from ovarian-related tissues and was produced using the Illumina HiSeq 4000 platform (GPL20301). and contains samples

from 9 controls and 8 POI patients. another gene expression dataset from ovarian-related tissues used to complement the transcriptomic analysis; however, specific platform and sample size details for this dataset were not fully available in the public metadata (10).

Normalization and Batch Effect Handling

Given that the four GEO datasets included in this study originated from different tissue sources (granulosa cells, cumulus cells, and follicular-fluid exosomes) and were generated using heterogeneous platforms (RNA-seq, miRNA-seq, and single-base methylation sequencing), the raw expression matrices were not merged. As a result, cross-platform batch correction was not appropriate, and each dataset was processed independently using the normalization method best suited to its technology. For the RNA-seq datasets (GSE201276 and GSE253684), normalization was performed using the median-of-ratios method implemented in the DESeq2 package, which accounts for differences in library size and sequencing depth. The lncRNA dataset (GSE158529) was processed using the same normalization approach. For the methylation dataset (GSE267641), analysis was carried out on the pre-processed β-values provided in the original GEO submission, as these values were already normalized by the authors' pipeline. Because the datasets were not combined at the count level, batch-effect correction tools such as ComBat or limma were not applied. Instead, all integrative steps were performed after differential expression analysis, by intersecting the resulting DEGs, DEMs, and DELs. This strategy avoids artificial biases that can arise from merging fundamentally different data types while still enabling meaningful biological integration across datasets.

Identification of Differentially Expressed Genes and Construction of Protein-Protein Interaction Network

Differentially expressed mRNAs (DEGs) from granulosa cell and cumulus cell samples, differentially expressed miRNAs (DEMs) in follicular fluid exosomal samples, and differentially expressed LncRNAs (DELs) from granulosa cells between POI patients and controls were identified using the DESeq2 R package(11). A significance threshold of $P < 0.05$ and $|\log_2 FC| > 1.0$ was applied for all differential expression analyses. Subsequently, the protein-protein interaction (PPI) networks for the identified DEGs were constructed using the STRING database, with an interaction confidence score cutoff > 0.4 considered statistically significant(12).

Functional enrichment analysis

To investigate the potential biological roles of granulosa cells, cumulus cells, and follicular fluid exosomes in Polycystic Ovary Syndrome (PCOS), Gene Set Enrichment Analysis (GSEA) was performed using the clusterProfiler R package. Furthermore, to predict the potential functions of these Differentially Expressed Genes (DEGs), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) enrichment analyses were conducted, also utilizing the clusterProfiler package. The GO analysis encompassed three distinct categories: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). A p-value < 0.05 was considered

statistically significant. To achieve a more robust functional prediction of the DEGs, GO and KEGG analyses were additionally performed using ClueGO software (v2.5.8) (13).

Construction of lncRNA–miRNA–mRNA ceRNA network

Based on differentially expressed genes (DEGs) and differentially expressed lncRNAs (DELs), we constructed lncRNA–miRNA–mRNA triplets. In this regulatory model, a miRNA can bind to a target mRNA to promote its degradation. Conversely, a lncRNA can bind to and sequester the miRNA, thereby inhibiting the miRNA's function and preventing mRNA degradation. To build these triplets, we utilized specialized bioinformatics tools and databases to predict and confirm the lncRNA-miRNA and miRNA-mRNA interacting pairs. The resulting network was visualized using the ggalluvial R package(14).

To construct the lncRNA–miRNA–mRNA triplets (forming a ceRNA network), we implemented a sequential bioinformatics approach. First, lncRNA–miRNA pairs were predicted by analyzing the differentially expressed lncRNAs (DELs) using the **ncori** database. Next, the target genes of these miRNAs were predicted by integrating information from three independent databases: miRDB,(15, 16) miRTarBase,(15) and TargetScan(17).

Finally, these predicted target genes were cross-referenced with our list of differentially expressed genes (DEGs). The overlapping genes, along with their corresponding lncRNA–miRNA pairs, were then used to build the final ceRNA network.

GO and KEGG enrichment analysis of the ceRNA network

To further understand the function of genes in the ceRNA network, we accomplished GO and KEGG path analyses with the clusterProfiler R package on our ceRNA network to predict the function of this network. p-value < 0.05 was considered statistically significant.

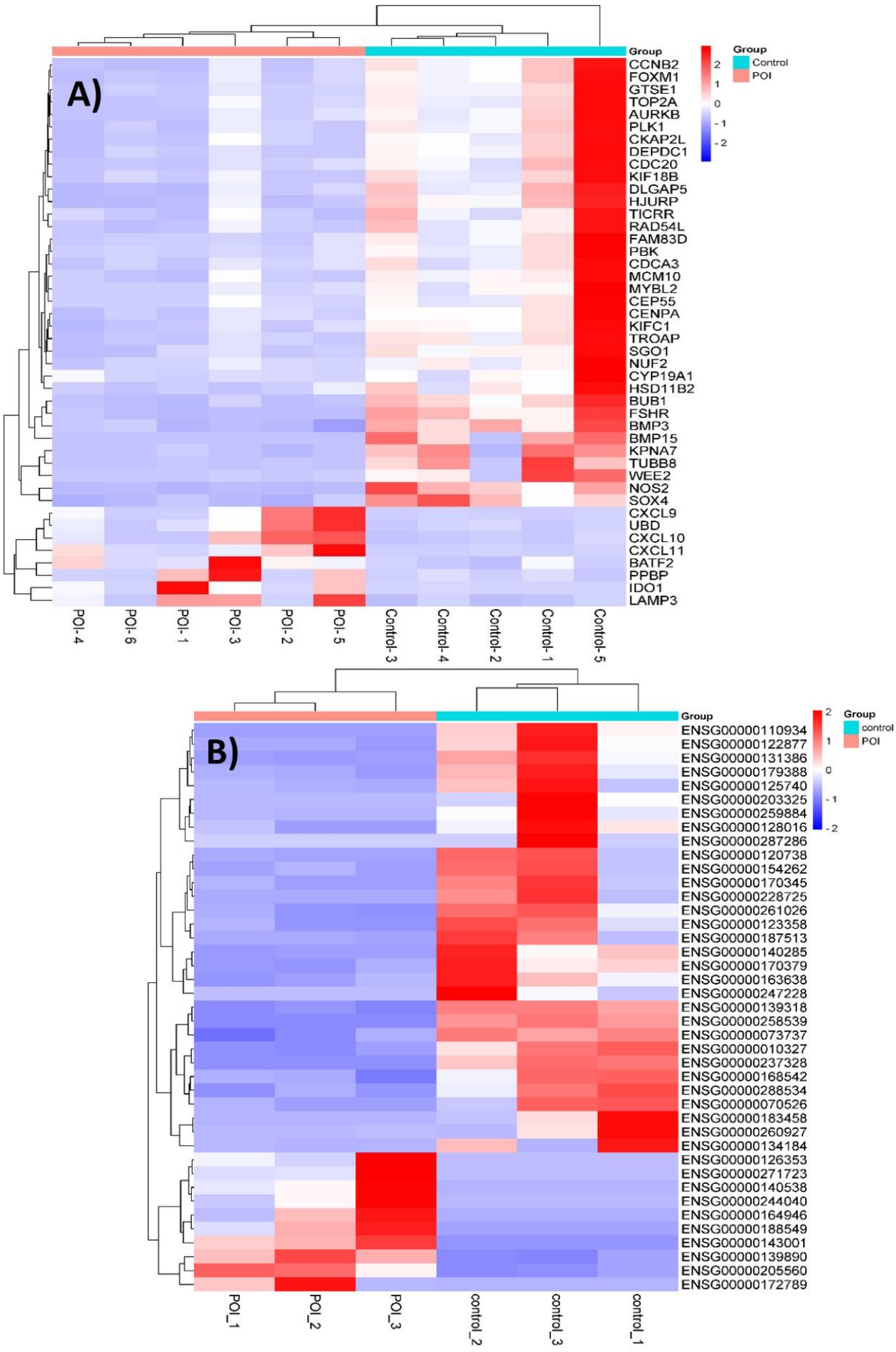
Results

Identification of differentially expressed genes

We identified 150 differentially expressed genes (DEGs; 123 up- and 27 down-regulated) from the GSE201276 dataset (granulosa cells), and 41 differentially expressed mRNAs from GSE267641 (cumulus cells). For clear visualization of coherent expression patterns, (Figure 2A) presents a clustered heatmap of a subset of these granulosa cell DEGs that showed strong co-expression relationships. Additionally, 53 differentially expressed miRNAs (DEMs) were identified in GSE253684, and 8 differentially expressed lncRNAs (DELs) were obtained from GSE158529. The complete sets of identified DELs, DEMs, and DEGs were utilized in subsequent bioinformatics analyses.

PPI network construction

To elucidate protein-protein interactions, we generated PPI networks for the differentially expressed genes (DEGs) from two distinct datasets using the STRING database. For dataset GSE201276, the resulting network comprised 44 nodes interconnected by 360 edges. Similarly, analysis of the GSE267641 dataset yielded a separate network consisting


A)
Group
Control
POI
CCNB2
FOXM1
GTSE1
TOP2A
AURKB
PLK1
CKAP2L
DEPDC1
CDC20
KIF18B
DLGAP5
HJURP
TICRR
RAD54L
FAM83D
PBK
CDCA3
MCM10
MYBL2
CEP55
CENPA
KIFC1
TROAP
SGO1
NUF2
CYP19A1
HSD11B2
BUB1
FSHR
BMP3
BMP15
KPNA7
TUBB8
WEE2
NOS2
SOX4
CXCL9
UBD
CXCL10
CXCL11
BATF2
PPBP
IDO1
LAMP3
POI-4
POI-6
POI-1
POI-3
POI-2
POI-5
Control-3
Control-4
Control-2
Control-1
Control-5
B)
Group
control
POI
ENSG00000110934
ENSG00000122877
ENSG00000131386
ENSG00000179388
ENSG00000125740
ENSG00000203325
ENSG00000259884
ENSG00000128016
ENSG00000287286
ENSG00000120738
ENSG00000154262
ENSG00000170345
ENSG00000228725
ENSG00000261026
ENSG00000123358
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ENSG00000247228
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ENSG00000073737
ENSG00000010327
ENSG00000237328
ENSG00000168542
ENSG00000288534
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ENSG00000134184
ENSG00000126353
ENSG00000271723
ENSG00000140538
ENSG00000244040
ENSG00000164946
ENSG00000188549
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POI_1
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control_2
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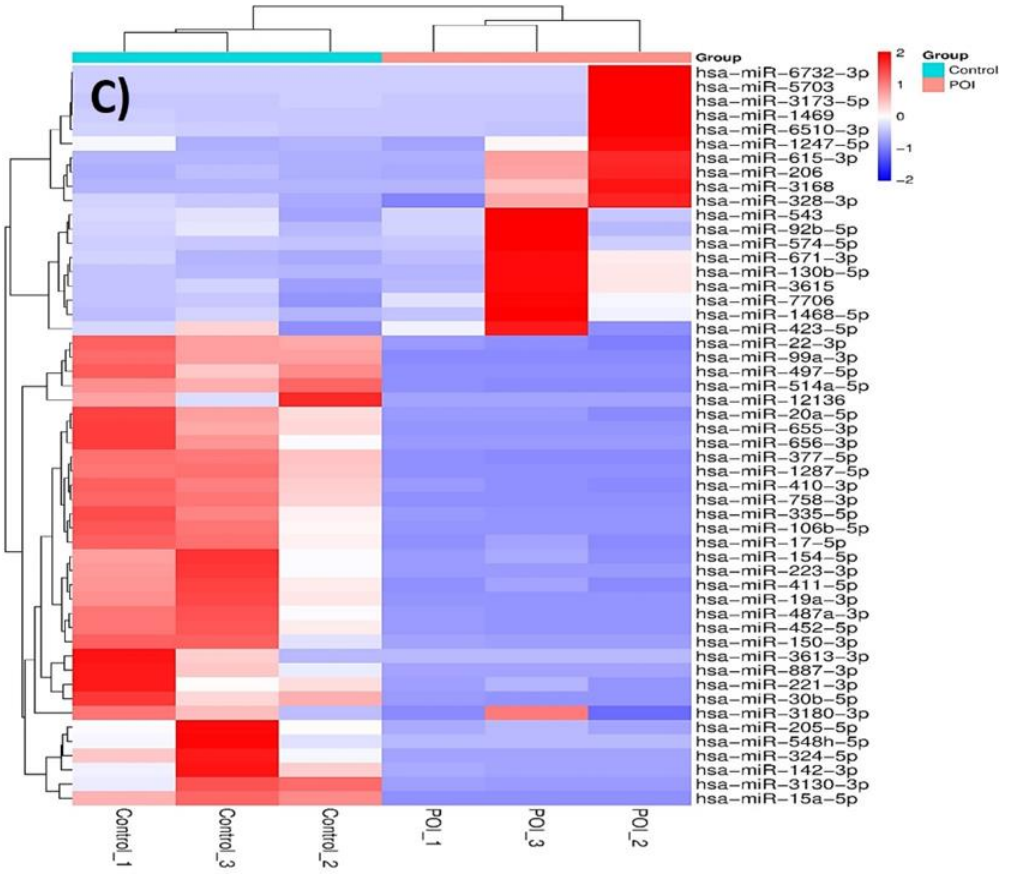
control_1



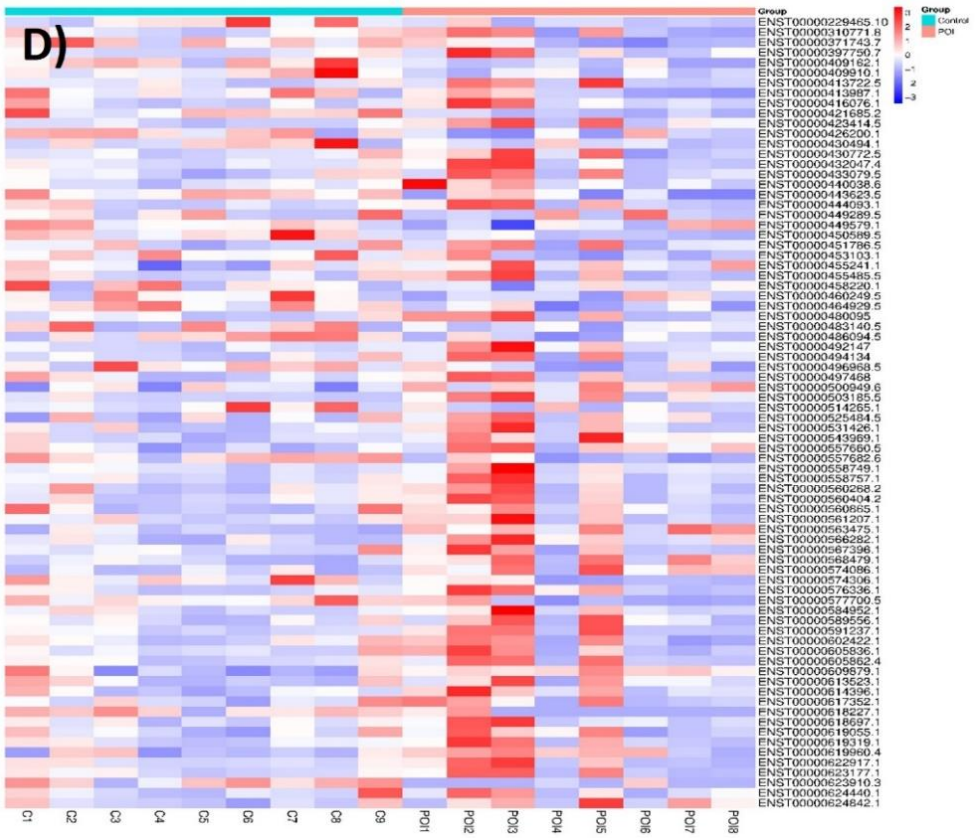


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Figure 2: (A) Heatmap of DEGs in Granulosa Cells (GSE201276): Shows the expression patterns of 150 DEGs (123 upregulated in red, 27 downregulated in blue) across POI and control samples. (B) Heatmap of DEGs in Cumulus Cells (GSE267641): Displays the expression patterns of 41 DEGs across sample groups. (C) Volcano Plot of Exosomal miRNAs (GSE253684): Visualizes 53 differentially expressed miRNAs in follicular fluid exosomes. (D) VolcanoPlotof lncRNAs (GSE158529): Shows the expression profile of 8 differentially expressed lncRNAs.

of 9 nodes and 27 edges (Figure 3). Both networks exhibited significant enrichment, with confidence levels corresponding to PPI enrichment p-values of less than 0.05. Within these networks, distinct subsets of up-regulated and down-regulated genes were identified for subsequent analysis.

Functional enrichment analysis

To gain a comprehensive understanding of the biological functions and pathways associated with the identified differentially expressed genes (DEGs), we performed Gene Ontology (GO) and KEGG pathway enrichment analyses separately for granulos cells (GSE201276) and cumulus cells (GSE267641). For granulosa cells, GO analysis revealed a highly significant enrichment of terms related to cell division and chromosome segregation in the Biological Process (BP) category, including 'nuclear division' and 'sister chromatid segregation' (Fig. 4 A). The Cellular Component (CC) terms were associated with chromosomal and spindle structures such as 'kinetochore' and 'spindle' while Molecular Function (MF) highlighted key binding activities like 'CXCR chemokine receptor binding' and 'microtubule binding'. This collective profile strongly suggests a profound dysregulation of the cell cycle. KEGG pathway analysis further substantiated this finding, showing significant enrichment in the 'Cell cycle' pathway, driven by genes like *BUB1* and *CDC20* (Fig.4B). Concurrently, pathways for 'Cytokine-cytokine receptor interaction' (involving *CXCL9*, *CXCL10*, *CXCL11*) and 'Platinum drug resistance' (involving *GSTA1*, *GSTA2*) were also prominent, indicating intertwined inflammatory and chemoresistance mechanisms. In contrast, the functional landscape of cumulus cells was distinctly different. Their GO profile was dominated by terms associated with cellular differentiation and development, such as 'skeletal muscle cell differentiation' and 'response to fibroblast growth factor' (Fig. 4C). CC terms pointed towards transcriptional regulation complexes ('RNA polymerase II transcription regulator complex') and RNA granules ('P-body'), while MF terms strongly emphasized DNA-binding transcription activator activity. This suggests a transcriptional program geared towards cell fate determination and post-transcriptional control. KEGG analysis of cumulus cells highlighted pathways primarily involved in intracellular signaling and disease processes (Fig. 4D). The 'MAPK signaling pathway' was a central hub, involving genes like *DUSP5* and *FOS*. In summary, the functional enrichment analyses reveal a clear dichotomy between the two cell types: granulosa cells are predominantly characterized by a signature of cell cycle disruption, whereas cumulus cells exhibit a profile rich in signaling regulation and differentiation processes. These distinct

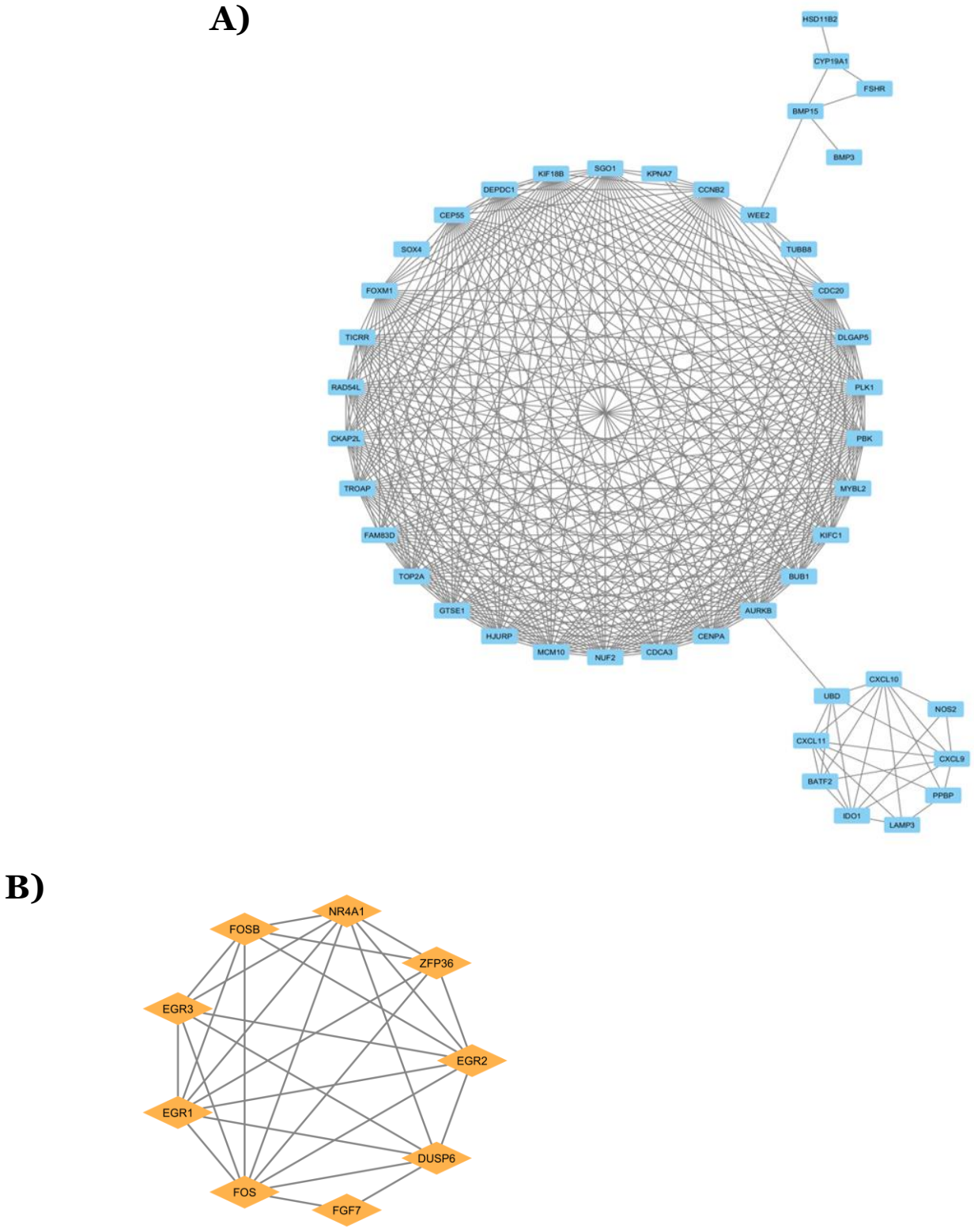

Figure 3. Protein-Protein Interaction (PPI) Networks. The PPI network for DEGs from (A) granulosa cells (44 nodes, 360 edges) and (B) cumulus cells (9 nodes, 27 edges). Hub genes are prominently displayed, illustrating the key interactive modules disrupted in POI.

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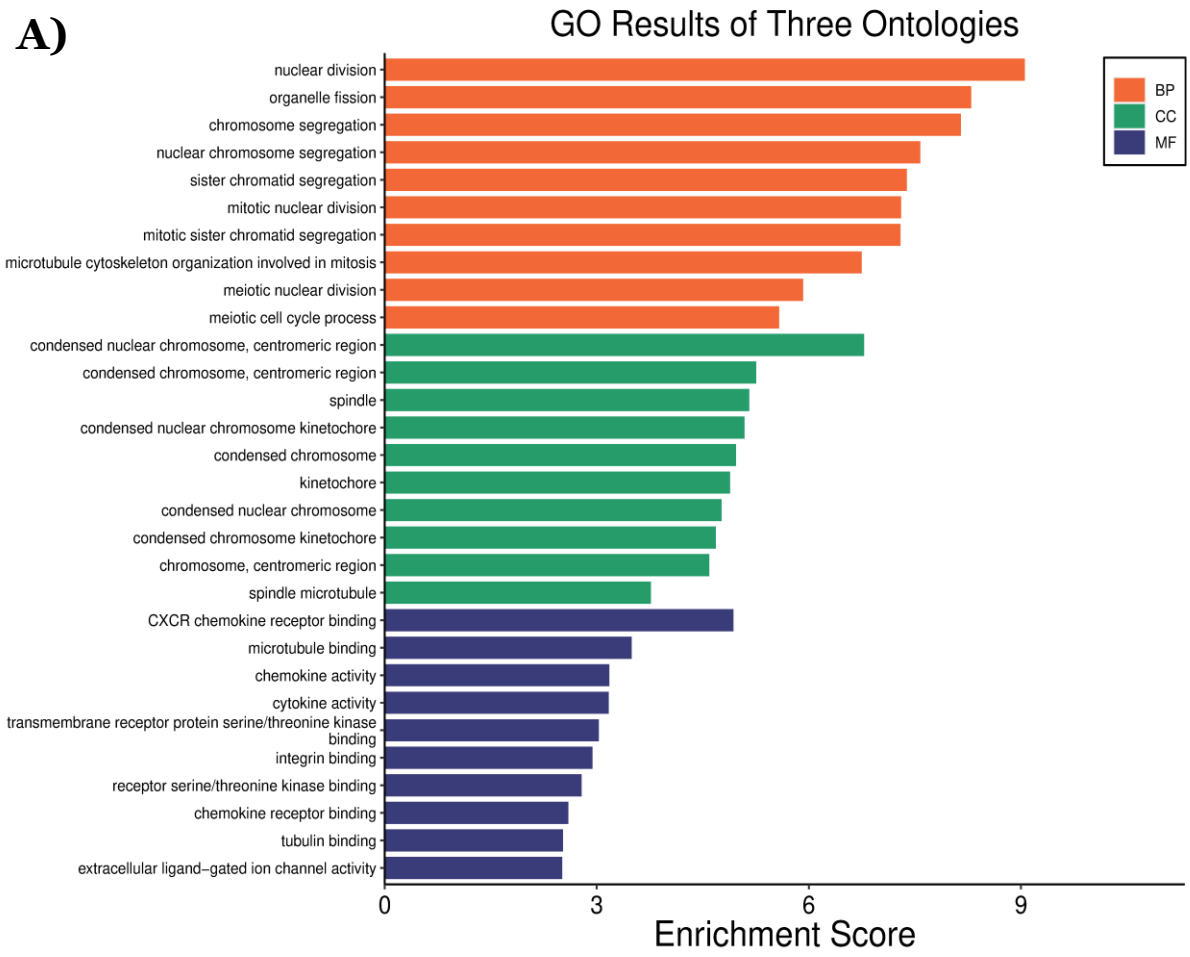
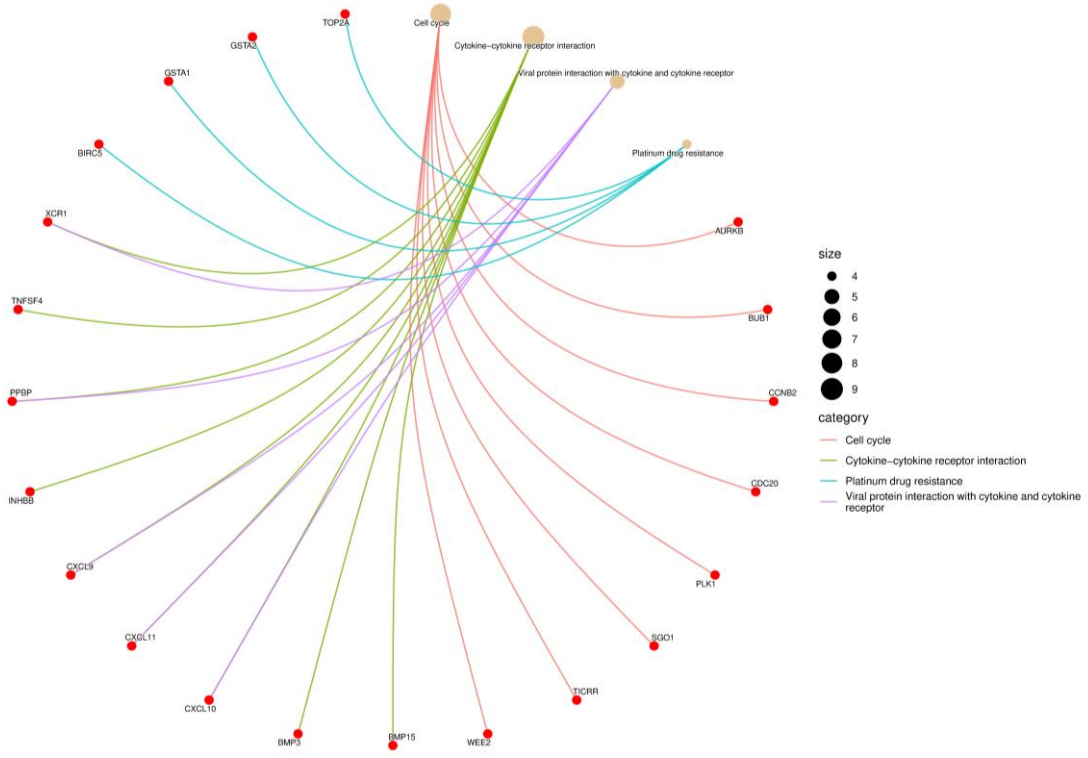
A)


GO Results of Three Ontologies
nuclear division
organelle fission
chromosome segregation
nuclear chromosome segregation
sister chromatid segregation
mitotic nuclear division
mitotic sister chromatid segregation
microtubule cytoskeleton organization involved in mitosis
meiotic nuclear division
meiotic cell cycle process
condensed nuclear chromosome, centromeric region
condensed chromosome, centromeric region
spindle
condensed nuclear chromosome kinetochore
condensed chromosome
kinetochore
condensed nuclear chromosome
condensed chromosome kinetochore
chromosome, centromeric region
spindle microtubule
CXCR chemokine receptor binding
microtubule binding
chemokine activity
cytokine activity
transmembrane receptor protein serine/threonine kinase binding
integrin binding
receptor serine/threonine kinase binding
chemokine receptor binding
tubulin binding
extracellular ligand–gated ion channel activity
BP
CC
MF
0
3
6
9
Enrichment Score

B)


TOP2A
Cell cycle
GSTA2
Cytokine–cytokine receptor interaction
GSTA1
Viral protein interaction with cytokine and cytokine receptor
BIRC5
Platinum drug resistance
XCR1
AURKB
TNFSF4
BUB1
PPBP
CCNB2
INHBB
CDC20
CXCL9
PLK1
CXCL11
SGO1
CXCL10
TCRR
BMP3
BMP15
WEE2
size
4
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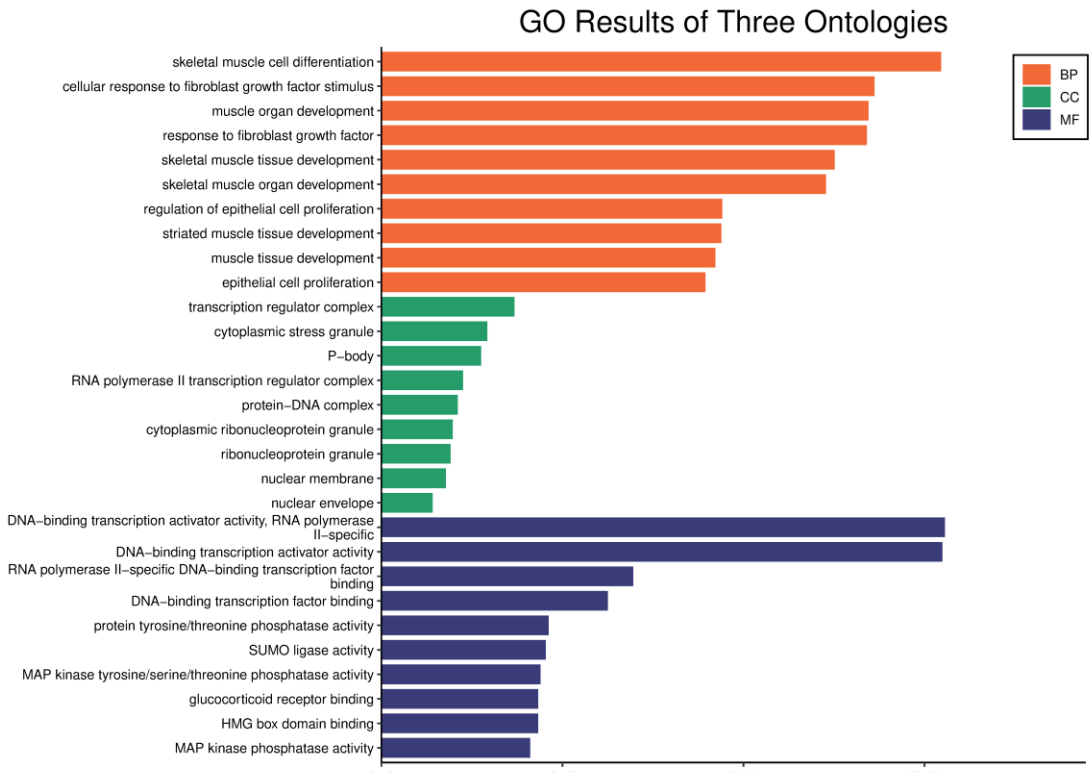
category
Cell cycle
Cytokine–cytokine receptor interaction
Platinum drug resistance
Viral protein interaction with cytokine and cytokine receptor

C)



D)

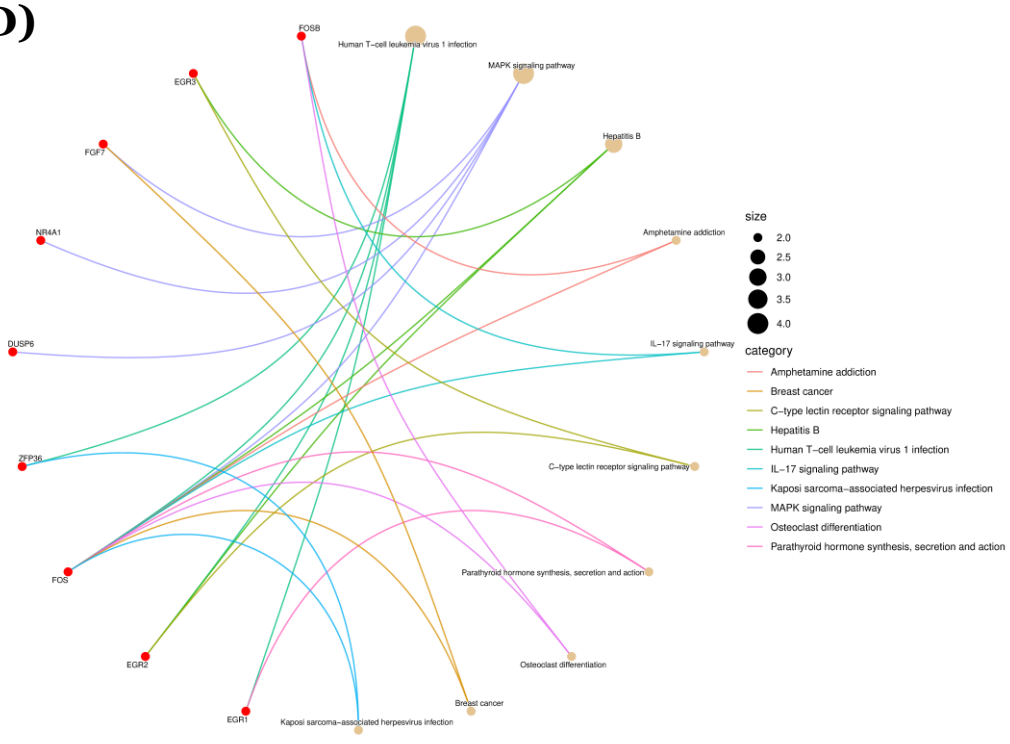


Figure4. Functional Enrichment Analysis of Granulosa Cell DEGs. (A) Bar plot of significantly enriched Gene Ontology (GO) terms (Biological Process, Cellular Component, Molecular Function). (B) KEGG pathway enrichment analysis. A cnetplot visualizing the genes associated with the key 'Cell cycle' and 'Cytokine-cytokine receptor interaction' pathways. Functional Enrichment Analysis of Cumulus Cell DEGs. (C) Bar plot of significantly enriched GO terms. (D) KEGG pathway enrichment analysis. A cnetplot illustrating the core genes within the 'MAPK signaling pathway' and other disease-related pathways.

molecular landscapes likely reflect their specialized roles within the ovarian follicle.

ceRNA network of lncRNA–miRNA–mRNA construction

The constructed competing endogenous RNA (ceRNA) network comprised 4 lncRNAs, 4 miRNAs, and 31 mRNAs. Within this network, a distinct expression pattern was observed: all four lncRNAs were downregulated, and among the four miRNAs, three were downregulated while one was upregulated. The majority of the mRNAs (29 out of 31) exhibited increased expression. These findings suggest that exosome-derived lncRNAs may contribute to the pathogenesis of Premature Ovarian Insufficiency (POI) by acting as molecular sponges for miRNAs, specifically via the miR-423-5p/miR-106b-5p/miR-452-5p/miR-3613-5p axis, thereby regulating the expression of their target mRNAs (Figure 5).

Genes in the ceRNA Network are Enriched in Key Pathways Regulating Stem Cells

KEGG pathway analysis performed on the 31 mRNAs comprising the core ceRNA network revealed significant enrichment in several key signaling pathways that directly regulate stem cell fate and function. The most notable among these were Signaling pathways regulating pluripotency of stem cells, Hippo signaling pathway, and TGF-beta signaling pathway. Notably, core network genes were integral components of these pathways; BIRC5 is a key regulator of the Hippo pathway, and INHBB—a ligand of the TGF-beta superfamily—contributes to both the TGF-beta signaling pathway and the Signaling pathways regulating pluripotency of stem cells. This finding indicates that the regulatory network uncovered by our study holds the potential to directly impact the function of the ovarian stem cell niche, beyond its disruptive effect on the somatic cell cycle.Although the KEGG pathways associated with stem cell regulation show p-values above the conventional significance threshold, they were retained due to their strong biological relevance to ovarian stem cell function. These pathways have been consistently reported in previous POI and ovarian biology studies, and their appearance among the network-derived genes suggests a non-random functional connection. Therefore, they are presented here to highlight potential mechanistic links that merit further investigation in future experimental or dataset-based validation studies.

Discussion

In this study, we constructed a multi-tissue ceRNA network to elucidate the potential molecular mechanisms underlying Premature Ovarian Insufficiency (POI). Our analysis revealed distinct yet complementary pathological signatures in granulosa cells and cumulus cells, and further identified a core exosome-mediated ceRNA axis that may play a pivotal role in POI pathogenesis.

The functional enrichment analysis painted a clear picture of cellular dysfunction in POI. In granulosa cells, the significant enrichment of cell cycle and chromosome segregation pathways, driven by hub genes like BUB1 and CDC20, points to a fundamental defect in cell proliferation and genomic stability. This mitotic catastrophe likely contributes to the accelerated depletion of the follicular reserve

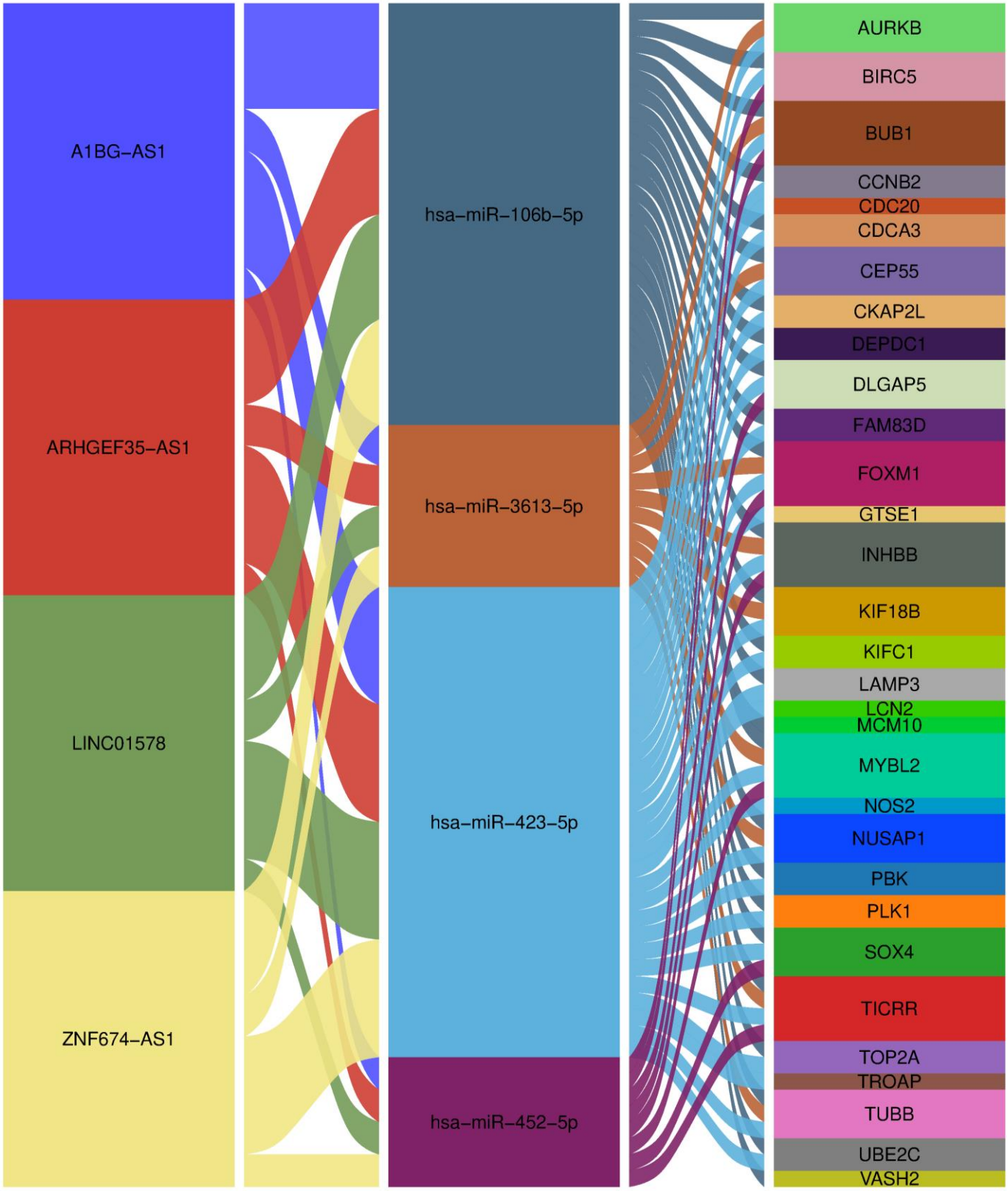


Figure5. The Core ceRNA Network in POI. The competing endogenous RNA (ceRNA) network integrating 4 lncRNAs , 4 miRNAs , and 31 mRNAs . This network posits that exosome-derived lncRNAs regulate mRNA expression by sponging miRNAs, revealing a potential central regulatory axis in POI pathogenesis.

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Table 1: The mRNAs in the ceRNA Network

Pathway ID	Pathway Name	Gene Symbol	P-Value
hsa04550	Signaling pathways regulating pluripotency of stem cells	INHBB	0.2659672
hsa04390	Hippo signaling pathway	BIRC5	0.2863426
hsa04350	TGF-beta signaling pathway	INHBB	0.2100324

Table2: Key genes from the ceRNA network are enriched in stem cell–related pathways (Key genes from the ceRNA network are enriched in stem cell–related pathways, indicating a potential disruption of the ovarian stem cell niche in POI. Although P-values are above 0.05, these pathways were retained for their biological relevance)

Gene	Predicted Biological Function	Interaction in ceRNA Network
TUBB	Cytoskeletal organization; spindle formation	Interacts with regulatory miRNAs and lncRNAs in cell-cycle control
TOP2A	DNA topology; chromosome segregation	miRNA–lncRNA competitive regulation
FOXM1	Cell-cycle progression; follicular growth	Targeted by miRNAs; regulated by lncRNAs
NUSAP1	Spindle assembly; microtubule binding	ceRNA-associated proliferation regulator
PLK1	Cell-cycle checkpoint; meiotic regulation	miRNA-mediated repression
KIF18B	Chromosome alignment; microtubule motor protein	Competes for shared miRNAs
KIFC1	Spindle pole focusing; oocyte division	lncRNA–miRNA–mRNA axis regulation
MYBL2	Cell-cycle control; proliferation	ceRNA-associated transcription factor
TICRR	DNA replication initiation	Interacts through shared miRNAs
SOX4	Differentiation; follicular development	Competing endogenous target
AURKB	Chromosome segregation; meiosis	Regulated by ceRNA interactions
TROAP	Spindle assembly; oocyte maturation	miRNA–lncRNA competitive binding
GTSE1	Cell-cycle regulation; DNA damage response	ceRNA-associated regulator
PBK	Mitotic kinase; proliferation	Competes for ceRNA miRNAs
CEP55	Cytokinesis; cell-cycle progression	miRNA–lncRNA axis
INHBB	Folliculogenesis; TGF-beta pathway	Ovarian regulator in ceRNA network
CDCA3	G1/S transition	Interacts with miRNAs
CKAP2L	Cytoskeletal dynamics	ceRNA-mediated role
DEPDC1	Cell-cycle progression	lncRNA–miRNA–mRNA regulation
DLGAP5	Spindle stability; mitosis	miRNA competitive binding
LAMP3	Stress response; apoptosis	ceRNA-associated stress gene
CCNB2	G2/M checkpoint; meiosis	miRNA competition
FAM83D	Spindle positioning	ceRNA-linked regulator
BUB1	Spindle checkpoint	miRNA-target competition
CDC20	APC/C activation; oocyte maturation	ceRNA-associated meiotic regulator
LCN2	Inflammation; ovarian signaling	miRNA–lncRNA regulation
NOS2	Nitric oxide signaling	ceRNA-associated metabolic regulator
UBE2C	Ubiquitination; cell-cycle progression	Competes for miRNAs
BIRC5	Apoptosis inhibition	ceRNA network apoptosis-related gene
MCM10	DNA replication initiation; replication fork stability	Interacts with regulatory miRNAs in ceRNA network
VASH2	Angiogenesis; ovarian follicular regulation	lncRNA–miRNA–mRNA axis involvement

, a hallmark of POI. Concurrently, the upregulation of inflammatory pathways such as 'Cytokine-cytokine receptor interaction' suggests a pro-inflammatory ovarian microenvironment that may further exacerbate granulosa cell apoptosis and dysfunction(18). In stark contrast, cumulus cells exhibited a profile centered on 'MAPK signaling' and 'cellular differentiation'. The downregulation of key regulators like *DUSP5* and *FOS* indicates a disruption in the finely-tuned signaling circuits that govern cumulus-oocyte communication(19). This dysfunction could impair oocyte maturation and quality, providing a mechanistic link between somatic cell defects and the reproductive failure observed in POI patients. Notably, our findings suggest a crucial paracrine dialogue between granulosa and cumulus cells that appears disrupted in POI. The observed cell cycle defects in granulosa cells may be influenced by impaired growth factor signaling originating from stressed cumulus cells, particularly under glucocorticoid regulation. Conversely, granulosa cells likely modulate cumulus function through specific growth factors such as BMP15 and BMP3, whose decreased expression we observed in POI patients. This bidirectional communication is essential for maintaining follicular integrity, and its disruption creates a vicious cycle where dysfunction in one cell type exacerbates pathology in the other, ultimately compromising follicle development and oocyte quality (20, 21). The most compelling finding of our study is the construction of a cohesive ceRNA network, anchored by four downregulated exosomal lncRNAs. The dominant pattern within this network—downregulated lncRNAs sponging downregulated miRNAs to relieve repression on upregulated mRNAs—suggests a coherent biological narrative. Specifically, we propose that the loss of exosomal lncRNAs (such as those we identified) in the follicular fluid fails to sequester miRNAs like miR-106b-5p and miR-452-5p. This leads to excessive activity of these miRNAs, which in turn represses target mRNAs critical for folliculogenesis. However, the net increase in mRNA expression in our network implies that the dominant effect is the inhibition of these repressive miRNAs by the lncRNAs, ultimately leading to the stabilization of pro-follicular genes. Recent evidence suggests that exosomal lncRNAs could offer new therapeutic opportunities for ovarian regeneration. Several studies using mesenchymal stem cell–derived exosomes have shown improvements in follicle numbers, hormone levels, and overall ovarian function in POI models, indicating that the RNA cargo of these vesicles plays an active role in repair. In this context, delivering beneficial lncRNAs through engineered exosomes—or silencing harmful ones using antisense oligonucleotides or siRNA-based approaches—may help support granulosa cell survival and restore follicular signaling. Although these strategies are still at the experimental stage, they highlight a realistic direction for developing RNA-based therapies for POI and underscore the need for further validation in ovarian cell systems and animal models (23 ,22). Our functional analysis provides direct support for this concept, revealing that the mRNA components of our core ceRNA network are significantly enriched in key pathways governing stem cell pluripotency and niche regulation, including the Hippo signaling pathway and Signaling

pathways regulating pluripotency of stem cells. Crucially, this mechanism bears a striking resemblance to processes governing stem cell fate (24). The miRNAs central to our network, particularly miR-106b-5p and miR-423-5p, are known regulators of self-renewal and differentiation in various stem cell populations, including ovarian germline stem cells (25). The dysregulation of this exosomal ceRNA axis could potentially disrupt the delicate balance between ovarian somatic cell function and the putative stem cell niche, impairing the homeostatic maintenance and regenerative capacity of the ovarian follicle pool. Therefore, our findings not only illuminate a novel ceRNA-mediated pathway 9in POI but also open an exciting avenue for future research: investigating whether targeting these exosomal lncRNAs could help to rejuvenate the ovarian microenvironment and potentially restore a functional stem cell niche, offering a groundbreaking therapeutic strategy for ovarian aging and infertility.

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