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SRC involves in lysosomal function and regulates ferroptosis in polycystic ovary syndrome

Tianmei Wang[1], Xin Chen[2] and Cong Li[1*]

Abstract

Background The pathogenesis of polycystic ovary syndrome (PCOS) is still unknown, so finding the molecular mechanisms of pathogenesis is crucial in PCOS.

Methods The GSE34526 dataset from the Gene Expression Omnibus (GEO) database was used to screen biomarkers in this study. KEGG enrichment analysis of GSE34526 was performed using Gene Set Enrichment Analysis (GSEA). The differentially expressed genes(DEGs) were screened and analyzed for lysosome-related genes. Subsequently, further KEGG and GO analyses were performed, and it was found that it was enriched in the ferroptosis pathway, and then the ferroptosis-related differential genes were obtained. The genes at the core position were obtained by the Protein-Protein Interaction(PPI) network. We then focused our attention on SRC and verified the differential expression of SRC in ovarian tissues of hyperandrogenemic, hyperlipemic and control groups, as well as the differences in conception rate and litter rate of each group by rat test.

Results GSEA analysis of the gene dataset GSE34526 revealed that LYSOSOME was significantly enriched in the PCOS group. There were 188 lysosome-related differentially expressed genes(LRDEGs) in granulosa cells from patients with PCOS, and 41 ferroptosis-related differentially expressed genes(FRDEGs). It was found that six of these genes, SRC, NCF2, SLC2A8, FTL, SLC2A6, SLC3A2, were present in all three datasets. SRC was the top ranked gene in the PPI network of FRDEGs.As verified by the rat model, the expression of SRC in the ovarian tissues of the hyperandrogenemic group was significantly higher than that of the control group (P=0.004) and the hyperlipemic group (P=0.002).

Conclusion SRC, as an important gene involved in lysosomal function and regulating ferroptosis, is expected to be a potential target for PCOS.

Keywords PCOS, Ferroptosis, Lysosome, Hub genes

Introduction

Polycystic ovary syndrome (PCOS) is a disorder characterized by oligo/anovulation, hyperandrogenism, and polycystic ovaries, and the syndrome can lead to a range of health problems, including infertility, insulin resistance, obesity, cardiovascular and psychological distress [1]. To date, the pathogenesis of PCOS remains unclear [2]. Therefore, there is an urgent need to find possible pathogenic mechanisms and thus more effective diagnosis and treatment of PCOS.

*Correspondence:
Cong Li
b05104@126.com
[1] Department of Gynecology, First Affiliated Hospital of Chongqing Medical University, Yuzhong District, Chongqing 400016, P.R. China
[2] Department of Gynecology and Obstetrics, The 958th Army Hospital of the Chinese People'S Liberation Army, Jiangbei District, Chongqing 400000, P.R. China

Lysosomes contain a variety of hydrolytic enzymes that promote cell renewal and reduce oxidative stress, mainly through autophagy [3]. Autophagy plays a role in insulin resistance (IR), oocyte development, metabolic abnormalities, anovulation, and follicular cell alterations in PCOS patients [4]. In addition, lysosomes can play a central role in regulating ferroptosis by coordinating the dynamic balance of iron in different cellular compartments [5]. Previous studies have demonstrated impaired iron metabolism in patients with PCOS, and the increased levels of iron death observed in granulosa cells are thought to be an important signaling pathway in the pathogenesis of PCOS [6].

Although a few studies have shown that ferroptosis and lysosomes are involved in processes associated with ovarian disease, the role of ferroptosis in PCOS needs to be further explored. In this study, we analyzed the genes related to ferroptosis and lysosomes in PCOS by bioinformatics methods for the first time to identify the pivotal genes to provide new directions for PCOS.

Results

GSEA of GSE34526

KEGG of GSE34526 was performed using GSEA to explore the pathway distribution of all gene expression data in PCOS patients and controls. The results showed that 122/173 gene sets are upregulated in PCOS patients and 61 gene sets are significant at FDR<25%. 51/173 gene sets are upregulated in the control group, and 2 gene sets are significant enriched at FDR<25% (Table S1).LYSOSOME was significantly enriched in the PCOS group with NES 2.04 (Fig. 1a), suggesting that it may play a key role in the pathophysiology of PCOS.

Identification of DEGs in GSE34526

Differential analysis of GSE34526 chip data revealed 3003 DEGs, including 2192 upregulated and 811 downregulated genes. These differential genes were clustered and analyzed as shown in the volcano diagram (Fig. 1b). Subsequently, using venn diagram, 188 LRDEGs were obtained (Fig. 1c). And a heatmap is presented in Fig. 1d.

Functional enrichment analysis

The results of KEGG enrichment of LRDEGs showed that these genes were most multiply enriched in asthma and most highly enriched in the phagosome and were enriched in many immune-related aspects, in addition, these genes were enriched in the ferroptosis pathway, demonstrating that lysosomal actions may be related to ferroptosis in patients with PCOS. The GO enrichment analysis consisted of three parts: biological process (BP), cellular component (CC), and molecular function (MF). In terms of BP, LRDEGs were primarily involved in peptide antigen assembly with MHC class II protein complex, antigen processing and presentation of exogenous peptide antigen via MHC class II, as

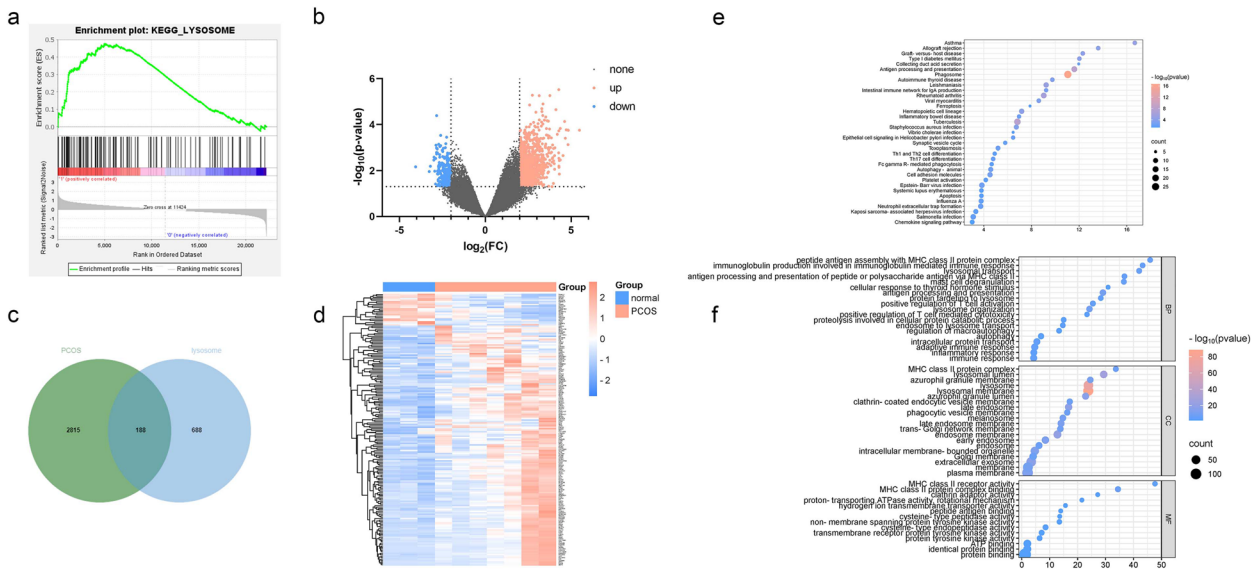

Fig. 1 Identification of the LRDEGs. **a** Differential gene enrichment in LYSOSOMOE between PCOS and control groups. **b** Volcano plot of genes in PCOS patients in GSE34526. Red indicates up-regulated genes, blue indicates down-regulated genes, and gray indicates genes not differentially expressed. **c** Venn diagram of DEGs and lysosome-related genes. **d** Heatmap of LRDEGs. Red represents high expression, and blue represents low expression. **e** KEGG enrichment analysis of the LRDEGs. **f** The top 20 significantly enriched GO terms in the category of BP, CC, and MF for the LRDEGs. The horizontal coordinate indicates the fold enrichment, the size of the dot indicates the number of genes enriched in the pathway, and the color indicates the FDR value (corrected *p*-value), with redder indicating more significant results

well as immunoglobulin production involved in immunoglobulin mediated immune response. Whereas, in terms of CC, the greatest fold enrichment was found in the MHC class II protein complex, and the highest enrichment was found in lysosome and lysosomal membrane. In terms of MF, LRDEGs were markedly enriched in MHC class II receptor activity and MHC class II protein complex binding (Fig.e-f).

FRDEGs

Since enrichment analysis revealed that LRDEGs were enriched in the ferroptosis pathway, we downloaded the ferroptosis genes from the FerrDb database and intersected the DEGs with them, and 41 overlapping FRDEGs were further identified (Fig. 2a).

KEGG enrichment of FRDEGs showed that these genes were significantly enriched in the fluid shear stress and atherosclerosis pathway, in addition to being most multiply enriched in the mitophagy pathway, and more highly enriched in the lipid and atherosclerosis and autophagy pathways (Fig. 2c-d).

PPI network analysis

To investigate the role of FRDEGs further, we uploaded them to STRING to construct a PPI network (Fig. 3a). Subsequently using the Cytoscape plugin (MCC), in the PPI network of FRDEGs, SRC, JUN, ALB, STAT3, KRAS, ZEB1, HMOX1, ATG7, ATM and HMGB1 are the top10 key genes, which are at the core of the regulatory network. Among them, SRC is the gene of top1, which may be an essential protein for the occurrence of ferroptosis in PCOS patients (Fig. 3b).

Single-gene GSEA pathway analysis of SRC

Based on the above results, we focused our attention on the SRC. In the previous KEGG and GO enrichment analyses we found that SRC is involved in fluid shear stress and atherosclerosis pathway, and lipid and atherosclerosis. In addition, SRC appears in the positive regulation of the apoptotic process and lysosomes.

To explore the potential functions of SRC, we carried out a single-gene GSEA-KEGG pathway analysis. SRC might be involved in arachidonic acid metabolism (NES=1.84) and lysosome (NES=1.24),

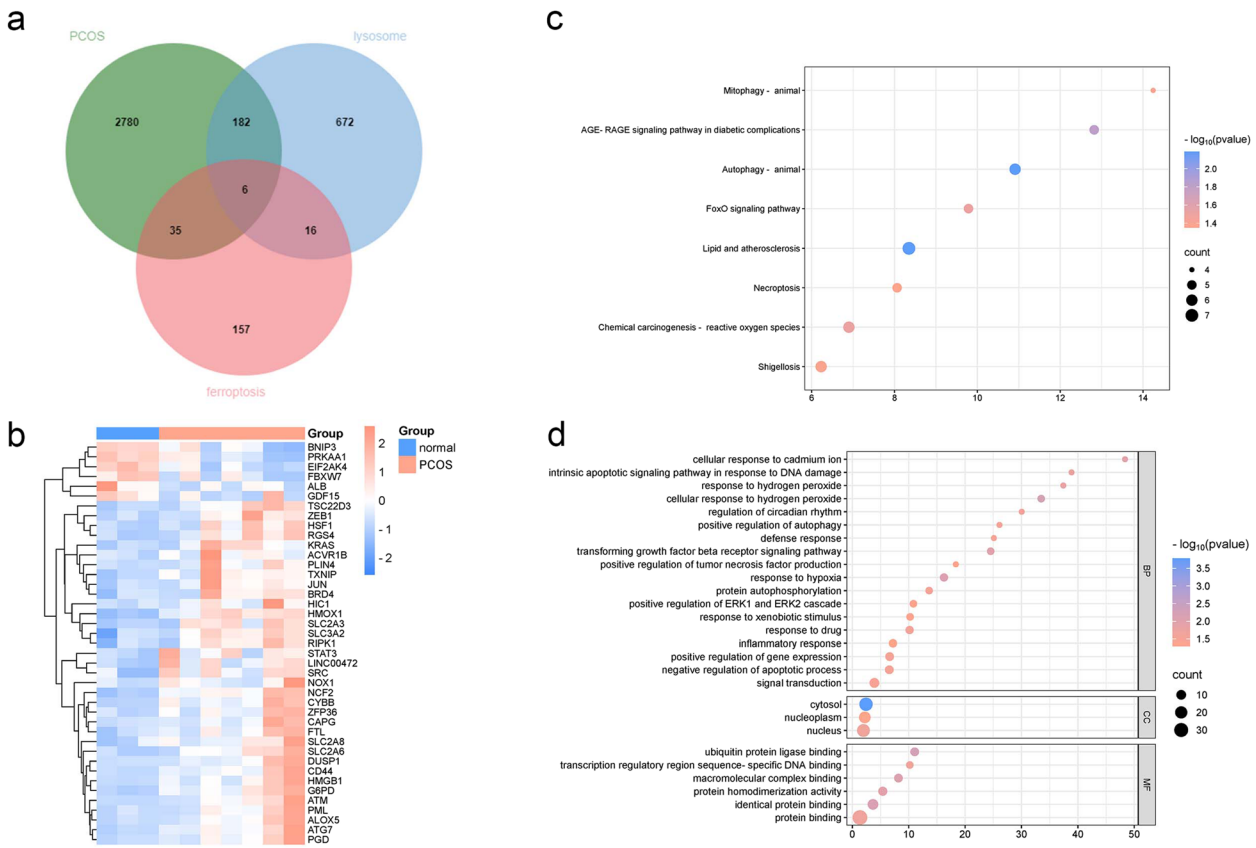

Fig. 2 Identification of FRDEGs. **a** Venn diagram of DEGs and ferroptosis/lysosome-related genes. **b** Heatmaps of FRDEGs. **c** KEGG enrichment analysis of the FRDEGs. **d** GO functional enrichment analysis for the FRDEGs

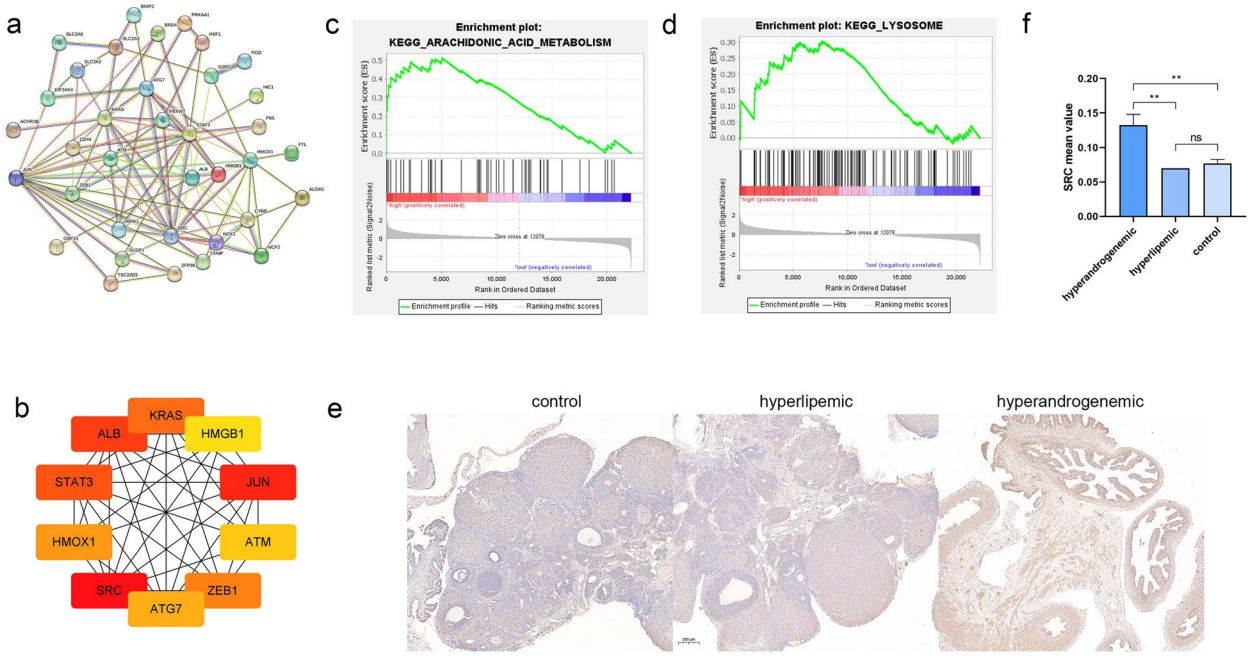

Fig. 3 SRC selection, function and expression. **a** PPI network of all the FRDEGs; genes with no connected dots are hidden. **b** Crosstalk between top 10 hub genes ranked by MCC algorithm and other FRDEGs. The deeper the color of the dot, the more advanced the rank order of the hub gene was. **c**-**d** GSEA for SRC. **e**-**f** SRC IHC images and expression analysis of 3 groups. ***p* <0.001

which further suggests the effect of SRC on lysosomal function in patients with PCOS (Fig. 3c-d).

SRC expression in the 3 groups

In order to further validate the above results, we used rat experiments for IHC of SRC in rat ovarian tissues. We measured the expression level of SRC in the ovaries of different groups of mice (Fig. 3e), and it showed that SRC had a higher mean value of SRC in the mice of the hyperandrogenemic group, which was statistically significant when compared with both the control group (P=0.004) and the hyperlipemic group (P=0.002) (Fig. 3f).

Comparison of conception rate and litter size among the 3 groups

The results showed that the conception rate of female rats fed with high-fat chow (4/9) was lower than that of the control group (6/9) but higher than that of the hyperandrogenemic group (2/9); the litter size (7/2) and conception rates of the hyperandrogenemic group were significantly lower than those of the other 2 groups (40/4 and 65/6) (Fig. 4). However, probably due to the small sample size, there was no statistical difference in the comparison of all groups.

Methods

Source of data

We downloaded a microarray dataset (GSE34526) from the GEO (https://www.ncbi.nlm.nih.gov/geo) to investigate differential gene expression in granulosa cells from PCOS patients and controls. The dataset includes gene expression profiles of 7 PCOS patients and 3 normal controls derived from the GPL570 sequencing platform.

GSEA

GSEA_4.3.2 (https://www.gsea-msigdb.org/gsea/index.jsp) was used to determine functional enrichment differences between the PCOS and control groups. Using the c2.cpkegg.v2022.1.Hs.symbols.gmt genome database, the number of permutation tests was 1000, normalized enrichment score-|NES|>1, normal p-value<0.05, false discovery rate- FDR q-value<0.25 for pathways that were significantly enriched.

DEGs selection

Comparing the gene expression patterns between disease group and the control group, DEGs were screened by the limma package using the GEO2R online analysis

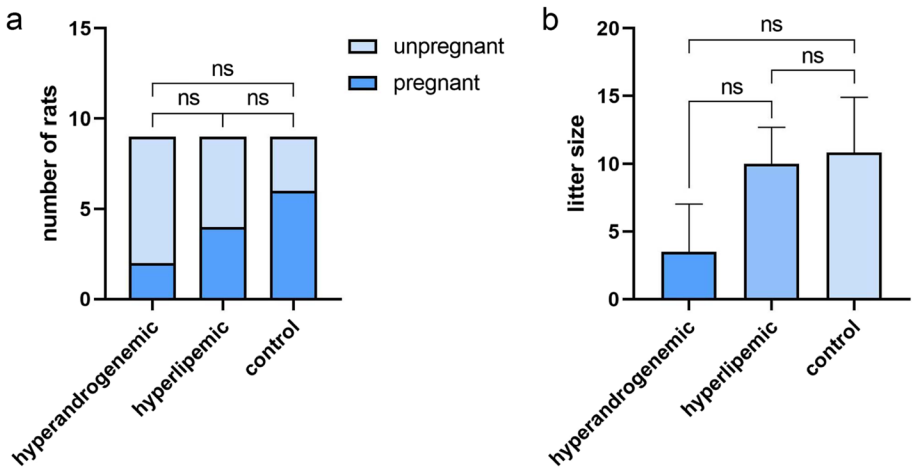

Fig. 4 Conception rate (**a**) and litter size (**b**) of rats in each group

tool (https://www.ncbi.nlm.nih.gov/geo/geo2r/) which is an R-based web application included in the GEO database, and standardize and correct all gene expression profiling microarray data and annotated the gene names. The thresholds for DEGs screening were |log2FC| >1 and p <0.05. P-values were adjusted to reduce the false positive rate using the Benjamini and Hochberg methods. Volcano maps were then plotted by https://www.bioinformatics.com.cn, an online platform for data analysis and visualization. In total, we used two datasets including 214 genes from the Ferroptosis Database (FerrDbV2) (http://www.zhounan.org/ferrdb/current/) and from the GO database (Gene Ontology) (http://geneontology.org/) 876 lysosome-associated genes, which were subsequently intersected with DEGs from GSE34526 to plot venn maps and heat maps based on DEGs by using BIOINFORMATICS.

Functional and pathway enrichment analyses

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed in Data for Annotation, Visualization, and Integrated Discovery (DAVID https://david.ncifcrf.gov/home.jsp), and FDR <0.05 was the inclusion criterion.

Protein-Protein Interaction (PPI) network analysis and hub gene identification

STRING database (https://cn.string-db.org/) was used to predict the interactions between proteins encoded by ferroptosis/lysosomes-related differentially expressed genes that may play an important role in PCOS. Significant PPIs were identified as interaction scores >0.4. The obtained results were imported into Cytoscape_v3.9.1 software for visualization and analysis. Hub genes with high connectivity, i.e. strongest correlation, in the PPI network were screened using the Cytoscape plug-in Maximal Clique Centrality (MCC) algorithm.

GSEA for single hub gene

To further explore the potential functions of the selected hub genes in PCOS, individual hub genes were analyzed using GSEA_4.3.2. In the dataset GSE34526, seven PCOS samples were divided into two groups, four in the low expression group and three in the high expression group, based on the median expression level of the hub genes. The c2.cpkegg.v2022.1.Hs.symbols.gmt was selected as the reference gene set. Pathways with |NES |>1, Normal p-value<0.05, and FDR q-value<0.25 were significantly enriched.

Experimental model of PCOS

Four-week-old female rats from the Experimental Animal Center of Chongqing Medical University (Chongqing, China) were housed in cages with ad libitum to water and food and a constant light/dark cycle (12 h/12 h). They were randomly divided into three groups, the hyperandrogenic group fed daily subcutaneous injections of testosterone propionate (TP) (1 mg/(100 g-d, Xianju Pharmaceutical) in the neck with standard rat chow; the high-fat group fed daily with 45% high-fat chow (brand: SYSE, product name: HFD (45% Kcal 1%fat)); the control group fed with standard normal rat chow. After the rats were fed for 50 days, they were anesthetized intraperitoneally with 2% sodium pentobarbital, and both ovaries were taken and sent for examination. All experimental procedures were conducted following the requirements of the Ethics Committee of Chongqing Medical University.

Observation of pregnancy rate and litter size

At the end of the intervention, the rats were fasted for 8 h. After sacrifice, the abdominal organs were fully exposed, the uterus was located, observed for number of pregnancy rats and the litter size.

Immunohistochemistry (IHC)

Paraffin-embedded treated ovarian tissues were taken and sectioned serially at 4 μm, and the paraffin sections were immunohistochemically examined using anti-c-SRC (Immunoway, YT1140, 1:300) according to the manufacturer's protocols, examined microscopically under a microscope, and captured the images. Stained areas were analyzed using Image J, and the MEAN values of three random fields of view were taken for each section.

Statistical analysis

Results were presented as mean ± standard deviation (SD), analyzed by SPSS 26.0 software, if the data conformed to the normal distribution and the variance was flush, one-way analysis of variance (ANOVA) was performed for comparison between multiple groups, and the difference was statistically significant with P <0.05.

Discussion

In order to find the pathogenesis of PCOS, we analyzed the differential genes related to lysosome and ferroptosis in PCOS for the first time, and further performed pathway enrichment analysis. Using PPI networks to obtain the central genes. We then focused on the SRC gene and verified the expression of SRC in rat ovarian tissue by IHC.

In this study, we first performed GSEA using gene expression profiling data from 7 PCOS patients and 3 control samples in GSE34526. We found that the PCOS group showed significant enrichment in the lysosomal gene set. Lysosomes function primarily through autophagy. In ovary, proper functional autophagy is required from oocyte production to follicle development and degeneration. Mitochondrial autophagy and autophagy cell death have been reported to be associated with mitochondrial division dysregulation, granulosa cell death, and follicle growth arrest in PCOS patients [7]. Li et al. found that androgen excess leads to the expression of genes related to the regulation of autophagy, which leads to the activation of autophagy and apoptosis in granulosa cells of PCOS patients, impairing ovarian function. Then we explored 188 LRDEGs in PCOS that were most multiply enriched in asthma. Previously Gabriella et al. proposed the definition of "asthma-PCOS overlap syndrome" denoting a condition with common features of both diseases [8]. Our results may indicate that abnormalities in lysosomal function commonly influence both diseases.

In addition, LRDEGs are enriched in immune-related aspects, including antigen binding to MHC class II protein complexes, antigen processing and presentation, and immunoglobulin production. Ovulation is dependent on local self-limiting inflammation. In women with PCOS, there is chronic systemic inflammation [9, 10], and the ovulatory process can be disrupted by pathologic chronic inflammation of the ovaries caused by IR and hyperandrogenemia [11, 12]. It has been suggested that Th1-type immunity dominates systemic immunity in PCOS patients and may be related to the ovarian mechanisms that lead to anovulation [13]. Among the hub genes obtained from the subsequent PPI network, previous studies have proposed that STAT3 can influence the inflammatory state and apoptosis of granulosa cell through the JAK1/STAT3 pathway [14]. HMGB1, highly expressed in PCOS ovaries, is a pro-inflammatory cytokine. ATG7 regulates autophagy and they are associated with IR [15]. In addition certain cytokines produced by Th1 can induce autophagy [16]. Also, lysosomes can play an important role in inflammation, antigen processing presentation [17]. Combined with the results in the KEGG analysis, we hypothesized that the production of cytokines such as IL-2 and IFN-γ by Th1 cells in patients with PCOS induced autophagy, which affected ovulation.

In addition to this, these genes are enriched in the ferroptosis pathway, suggesting that the role of lysosomes in PCOS patients may be associated with ferroptosis. Ferroptosis is recognized as a form of autophagy-dependent cell death [18]. Lysosomes can play a central role in regulating ferroptosis by coordinating the dynamic balance of iron in different cellular compartments [5]. Iron can either be found in lysosomes leading to Lysosomal membrane permeabilization (LMP) and release of contents to the cytoplasm with subsequent cellular damage leading to lysosome-dependent cell death (LCD), or released from ferritin-increasing intracellular iron content and subsequently leading to oxidative damage via the Fenton reaction [19]. However its link to PCOS is still unclear. Several previous studies have concluded that serum ferritin levels are elevated in patients with PCOS, indicating increased body iron stores [20]. Potential link between abnormal iron metabolism and oxidative stress-mediated PCOS, which is strongly associated with IR, decreased glucose tolerance, and the development of diabetes mellitus [21]. So subsequently we screened 41 FRDEGs in GSE34526. FRDEGs are involved in the positive regulation of fluid shear stress and atherosclerotic pathways, lipids and atherosclerosis, autophagy, and apoptotic processes. Previous studies have also shown that patients with PCOS have significantly higher levels of triglyceride

to high-density lipoprotein (HDL) cholesterol ratios and are at higher risk for atherosclerosis, cardiovascular disease [22]. In addition, endothelial cell ferroptosis is an initiating factor in atherosclerosis [23]. Of the 10 hub genes we obtained through the PPI network, STAT3 has been found to affect metabolism and fertility in PCOS mice by regulating ferroptosis [24]. IL-22 can directly improve ovarian follicular development and ovulation through its classical downstream molecule STAT3 [25]. KRAS has been found to mediate cancer cell-macrophage communication through autophagy-dependent ferroptosis [26]. ZEB1, a transcription factor of the epithelial-mesenchymal transition (EMT) program, can operate ferroptosis in cancer cells by regulating lysophosphatase expression [27]. HMOX1 upregulation is involved in ferroptosis in the development of diabetic atherosclerosis [28]. ATM can orchestrate ferritin autophagy and ferroptosis by phosphorylating NCOA4 to innervate unstable free iron in the cell [29]. However little is known about the effects of the aforementioned pivotal genes on PCOS patients, which needs to be further explored.

SRC appeared in both ferroptosis and lysosome-related differential genes and also appeared as a pivotal gene in the ferroptosis-related top1 in the PPI interaction network. Therefore, we focused on SRC gene for further exploration. SRC is known as a non-receptor tyrosine kinase that regulates cell growth, proliferation, differentiation, and apoptosis, etc. SRC interacts with estrogen receptors and progesterone receptors [30, 31]. Some researchers have also found that knockdown of SRC impairs follicular development and reduces the number of pre-antral and antral follicles [32]. We verified the high expression of SRC in the PCOS model by IHC. Therefore, we subsequently performed single-gene GSEA enrichment analysis of SRC and showed that the enriched genome in the high-expression group contained lysosomes (NES=−1.25), which further suggests the impact of SRC on lysosomal function in PCOS patients. In addition arachidonic acid (AA) metabolism (NES=−1.84) was downregulated in the high-expression group. During ferroptosis, iron can generate ROS via the Fenton reaction, which can react with polyunsaturated fatty acids (PUFAs) on lipid membranes, especially and most readily with arachidonic acid to induce lipid peroxidation, leading to disruption of the lipid bilayer [33]. Thus our results suggest that in granulosa cells of PCOS patients, SRC is involved as an important gene in down-regulating AA metabolism and inducing lipid peroxidation, leading to ferroptosis. Previous studies in rat models have found that PCOS rats have higher serum AA levels but lower ovarian tissue AA levels, and significantly higher levels of the AA metabolite prostaglandins produced via the cyclooxygenase (COX) pathway [34]. There was an elevation in AA metabolites in follicular fluid of PCOS patients [35]. This indicates that the local AA content in ovarian tissue is different, which affects ovulation in PCOS. However, further detailed studies are needed.

To further explore whether SRC expression affects follicular development and ovulation. We compared the conception and litter rates of different groups of rats. Due to the small sample size, the results were not statistically different, but we can still see a certain trend. As the difference in litter size between the hyperlipemic group and the control group was small, while the hyperandrogenemic group not only had a low pregnancy rate but also had a significantly lower litter size than the control group. Therefore, we believe that the hyperandrogenemic state has an inhibitory effect on both follicular development and ovulation in the ovary, and the hyperlipemic state affects more ovulation than follicular development. In previous studies, AMH is over-expressed in PCOS due to excessive intrinsic androgen, which make GCs hypersensitive to FSH, resulting in excessive growth of preantral follicles [36], hyperandrogenism has been shown to affect follicular development and ovulation may be caused by impaired steroid hormone synthesis and lipid metabolism [37], but a number of studies have demonstrated that mice on a high-fat diet not only have affected primordial follicle numbers but also accelerated ovarian follicular development and follicular loss [38]. High-fat diet can affect the expression of genes associated with impaired estrous cycle, depletion of ovarian reserve and normal ovulatory function [39]. Moreover, Endothelin 2 (Edn2), a key gene for ovulation, is dysregulated throughout the estrous cycle by high-fat diet and can lead to ovulatory dysfunction [40]. Kiss1 mRNA and kisspeptin peaks in the ovary during proestrus and its expression was reduced by high-fat diet [41]. Although we didn't get the expected results, our study shows that although hyperandrogenemic can influence lipid metabolism, there are other factors that further influence follicular development and ovulation.

However, our study also has some limitations. First, the sample size of the dataset we chose was small. Second, due to the enrichment method, there may be false negatives, so other neighboring genes still need to be investigated. Third, the sample size in the final experimental validation was small and due to the heterogeneity of PCOS, it was hard to create a single animal model expressing all PCOS features. Therefore, further studies are still needed to explore the function of hub genes to help find the pathogenesis and treatment of PCOS.

Conclusion

In conclusion, our screened LRDEGs and FRDEGs in PCOS patients by bioinformatics analysis identified SRC as a potential regulator of lysosomal abnormality and ferroptosis response in PCOS patients, and validated it by rat model, and found some new directions for future PCOS research.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13048-025-01637-y.

Supplementary Material 1.

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Authors' contributions
All authors participated in the conceptualization and design of the project. Tianmei Wang performed the bioinformatics analysis and wrote the manuscript, Xin Chen performed the experiments, and Tianmei Wang and Xin Chen analyzed the data. Cong Li improved the manuscript. All authors read and approved the final manuscript.

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Data availability
Data is provided within the manuscript.

Declarations

Ethics approval and consent to participate
This study was reviewed and approved by the Ethics Committee of Chongqing Medical University (Approval number: IACUC-CQMU-2024-0229).

Consent for publication
Written informed consent for publication has been obtained from all necessary person(s).

Competing interests
The authors declare no competing interests.

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