RESEARCH ARTICLE

Repurposing new drug candidates and identifying crucial molecules underlying PCOS Pathogenesis Based On Bioinformatics Analysis

Zeinab Dehghan[1,2] · Samira Mohammadi-Yeganeh[2,3] · Marzieh Sameni[1,2] · Seyed Amir Mirmotalebisohi[1,2] · Hakimeh Zali[4] · Mohammad Salehi[2,3]

Abstract

Backgrounds Polycystic ovary syndrome affects 7% of women of reproductive ages. Poor-quality oocytes, along with lower cleavage and implantation rates, reduce fertilization.

Objective This study aimed to determine crucial molecular mechanisms behind PCOS pathogenesis and repurpose new drug candidates interacting with them. To predict a more in-depth insight, we applied a novel bioinformatics approach to analyze interactions between the drug-related and PCOS proteins in PCOS patients.

Methods The newest proteomics data was retrieved from 16 proteomics datasets and was used to construct the PCOS PPI network using Cytoscape. The topological network analysis determined hubs and bottlenecks. The MCODE Plugin was used to identify highly connected regions, and the associations between PCOS clusters and drug-related proteins were evaluated using the Chi-squared/Fisher's exact test. The crucial PPI hub-bottlenecks and the shared molecules (between the PCOS clusters and drug-related proteins) were then investigated for their drug-protein interactions with previously US FDA-approved drugs to predict new drug candidates.

Results The PI3K/AKT pathway was significantly related to one PCOS subnetwork and most drugs (metformin, letrozole, pioglitazone, and spironolactone); moreover, VEGF, EGF, TGFB1, AGT, AMBP, and RBP4 were identified as the shared proteins between the PCOS subnetwork and the drugs. The shared top biochemical pathways between another PCOS subnetwork and rosiglitazone included metabolic pathways, carbon metabolism, and citrate cycle, while the shared proteins included HSPB1, HSPD1, ACO2, TALDO1, VDAC1, and MDH2. We proposed some new candidate medicines for further PCOS treatment investigations, such as copper and zinc compounds, reteplase, alteplase, gliclazide, Etc.

Conclusion Some of the crucial molecules suggested by our model have already been experimentally reported as critical molecules in PCOS pathogenesis. Moreover, some repurposed medications have already shown beneficial effects on infertility treatment. These previous experimental reports confirm our suggestion for investigating our other repurposed drugs (in vitro and in vivo).

Keywords Systems biology · Polycystic Ovary Syndrome · Protein–protein interaction network · Infertility · Drug

✉ Mohammad Salehi
  m.salehi@sbmu.ac.ir

[1] Student Research Committee, Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[2] Cellular & Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[3] Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[4] Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Introduction

Polycystic ovary syndrome (PCOS) is considered a heterogeneous disorder and metabolic dysfunction reported in seven percent of women worldwide in their reproductive ages [1]. Around 45 percent of the infertility cases with unknown causes have PCOS-related morphology associated with ovarian dysfunction or hyperandrogenism. High androgen on PCOS cells affects the incomplete decidual transformation of endometrial cells [2, 3]. Some abnormalities are common in PCOS patients, including (i)-hyperandrogenism, (ii)-polycystic ovary, (iii)-reduced fecundity, (iv) hyperinsulinemia, and (v)-impaired GnRH [4]. It has been reported that some pathways, such as gonadotrophin hormone action, steroid hormone synthesis, and insulin-signaling pathway, play essential roles in PCOS pathogenesis [5–7].

In PCOS patients, treatments are chosen based on specific patient manifestations and are individualized. At present, some drugs have been approved by the US Food and Drug Administration for usage in PCOS. These drugs include metformin, spironolactone, pioglitazone, letrozole, clomiphene, acarbose, and rosiglitazone [8]. These drugs have been reported to be somewhat effective in treating PCOS by affecting oocyte maturation and ovulation abnormalities. The deciphering of their exact pharmacodynamics and pharmacokinetics needs further investigation.

The PCOS mechanism seems to be an enigmatic problem. New genomics and proteomics data, obtained from omics techniques, has brought tremendous information about its molecular pathology, and yet the deciphering of its exact mechanism seems to demand rigorous efforts. Newly, various text-mining strategies and methods have been applied to numerous different molecular biology and medicine tasks, such as drug discovery and molecular study of the disease [9, 10]. Recently, systems biology has improved our understanding of drug-protein interactions, and different networks of protein–protein interactions (PPI) have been used to predict the mechanisms of drug effects. Several studies have used various PPI networks to decipher underlying molecular mechanisms behind different human conditions [11]. Several others have used PPIs to clarify the molecular mechanisms of responses to drugs [12–15].

More recently, shared proteins among some associated diseases have been used to deepen our understanding of their molecular pathology in more detail [11]. Shared proteins between drug-related protein networks and disease PPI networks can help obtain new insights into the disease's molecular pathology. It helps elucidate the essential proteins, which can probably be used to prevent, diagnose, and drug design.

In this in-silico study, we pursue two different goals. We will first analyze the PCOS protein–protein interactions (PPI) to identify the PCOS pathogenesis's essential molecular mechanisms. We will then determine the significant interactions between the drug-related proteins (text-mined) and the PCOS-related protein subnetworks. Using this method will probably help us gain a more in-depth insight into the PCOS molecular mechanisms using the novel in-silico approach. Second, we will repurpose new drug candidates for PCOS treatment for further experimental studies since most current infertility medications are symptom-based, and investigations for better treatments coping with PCOS metabolic and reproductive abnormalities seem necessary [16]. In this study, we will first gather data on proteins related to PCOS to achieve the goals. We also will collect information on drugs involved in ovulation. We will then construct the PPI networks of interactions among the PCOS-related proteins and drug-related proteins to deepen our understanding of the PCOS molecular mechanism. After determining the crucial molecules using the analysis of PCOS PPI and PCOS drug-related protein networks, we will repurpose new drug candidates using the identified essential molecules.

## Methods

Design of the study

Recently bioinformatics approaches have been used to predict the molecular mechanisms behind some reproductive diseases [28, 29]. This study has used a novel bioinformatics approach to investigate the relations between the infertility drug-related proteins and the PCOS PPIN. The graphical workflow is represented in Fig. 1.

Data collection

In the current study, we extracted data from 16 proteomics papers available in PCOSBase (Supplementary Table S1). PCOSBase is a manually curated medical database. It is compiled from different genes and protein expression research papers and nine databases (including Disease [10], GWAS Catalog [17], MalaCards [18], GWASdb [19], Online Mendelian Inheritance in Man (OMIM) [20], Disease and Gene Annotation (DGA) [21], PhenomicDB [22], DisGeNet [23], and the Human Gene Mutation Database (HGMD) [24]). In sum, we obtained 168 up and down-regulated proteins in PCOS patients compared to the normal (Table S1). These studies have identified the differences in the amount of protein using mass spectroscopy, MALDI-TOF–MS, or LC–MS techniques. The investigated tissues included the ovary, granulosa cells, and follicular fluid.

Due to the significant role of the oocyte maturation and ovulation abnormalities in PCOS pathology, we selected

Fig. 1 Workflow. A graphical overview of the workflow
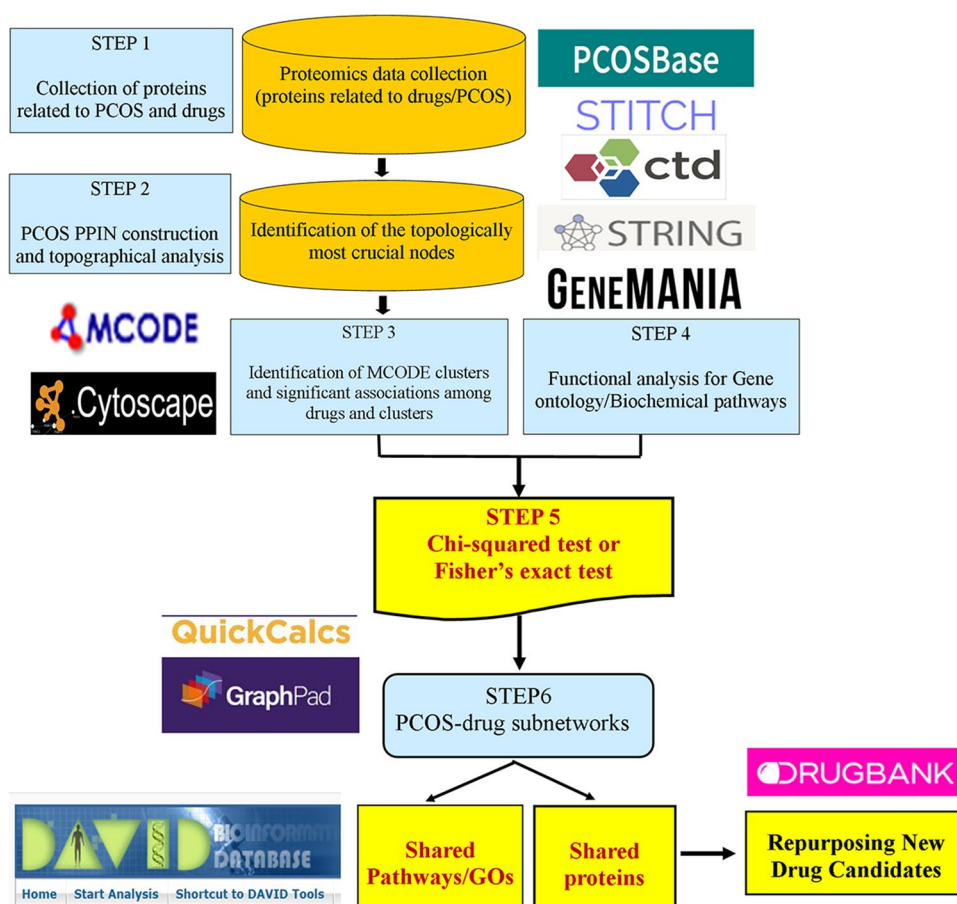
the clinical drugs used to treat infertility in PCOS that target these abnormalities (according to the literature). These drugs included acarbose, clomiphene, letrozole, metformin, pioglitazone, rosiglitazone, and spironolactone [8]. The proteins related to these drugs (experimental text-mined relation) were then obtained using two experimental databases (including CTD and STITCH) (Table S2). The comparative Toxicogenomics Database (CTD) reports the experimented text-mined relations among diseases, drugs, genes, chemicals, environmental exposures, and pheno-types [25]. The STITCH database also represents the different types of interactions between drugs and proteins [26].

Construction of PCOS PPI Network

PCOS Protein–protein interaction network (PPI) was constructed using the Protein–protein interaction map available in the STRING database (https://string-db.org/). STRING is a database that uses experimental data and computational prediction methods to predict protein–protein interactions. We used Cytoscape 3.5.1 software to visualize the PPI network and further analyses [40].

Topological analysis and functional enrichment of hubs

Cytoscape Network Analyzer was used to analyze the topological parameters, including Degree and Betweenness Centrality (BC). Nodes with higher degree values were considered as the hubs. Nodes with higher Betweenness Centrality were considered as bottlenecks [38]. Functional analysis was then performed on the top ten hub nodes using GeneMA-NIA. GeneMANIA is a tool for gene function prediction [41].

Construction of PPI subnetworks

The Molecular Complex Detection (MCODE) app was used to determine the highly interconnected PCOS network regions, called MCODE clusters. The MCODE identifies the densely connected subnetworks from a vast interaction network. Subnetworks with a score of interaction higher than 2.0 and at least two nodes were identified as significant. (The MCODE parameters settings included Degree Cutoff = 2, Node Score Cutoff = 0.2, K-Core = 2, and Max-Depth = 100.) [27].

The number of shared proteins between the PCOS PPI subnetworks and drug-related proteins was then investigated to determine the significant relations between different MCODE motifs and drugs. The chi-squared test or Fisher's exact test (taking into account the requirements of the Chi-square test) were used to identify statistically available significant relations between the drugs and the subnetworks obtained from the PCOS PPI network (p-value < 0.05). The chi-squared test only works for random data and independent samples, and its sample size must be large enough [28]. Fisher's exact test was used when the chi-square conditions were not met. The 2×2 contingency tables have been used to analyze the statistically significant relations using the GraphPad online calculator [29]. This method can be used to evaluate the associations between diseases or assess the significance of a relationship between disease and their disease-associated genes [11, 30, 31]. The associations were evaluated using a, b, c, and d numbers shown in Table 1.

The PCOS subnetworks significantly related to the PCOS drugs were then classified as PCOS-drug subnetworks and were selected for the biochemical pathway and biological process enrichment analysis to identify shared pathways and processes between PCOS and the drugs.

Table 1 2×2 contingency table. Fisher's Exact test with values: a, b, c, and d

	Shared between the drug and the PCOS-PPI network	Non-shared between the drug and PCOS-PPI network	
In subnetwork	a	b	a+b
Outside subnetwork	c	d	c+d
	a+c	b+d	n*

Note: n* was the number of all proteins available in the PCOS-related PPI network

Biochemical Pathway and biological process enrichment analysis

Biochemical pathway and biological process enrichment analysis were carried out using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/) [32] according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database [33]. DAVID provides functional annotation tools for analyzing biological themes for lists of genes [32]. This database was used to identify pathways and biological processes shared between subnetworks and drugs. Shared Biochemical Pathways were determined between the medicines and PCOS subnetworks.

Drug-protein interaction analysis

We constructed a collection of proteins containing two main groups. First, we selected the most crucial molecules of the PPI (hub-bottlenecks) (Table 2). Second, we added the shared molecules between PCOS MCODE clusters and the drug-related proteins to the collection (Table 3). We then used the DrugBank database (https://go.drugbank.com/) to repurpose new drug candidates for the crucial proteins. The new recommended drugs were selected from the previously approved drugs readily available in DrugBank. Finally, we visualized the interaction network between new repurposed drugs and the selected proteins using Cytoscape 3.5.1 software.

Results

PCOS Protein–protein interaction network

The number of differentially expressed proteins in PCOS patients was 168. (107 up-regulated proteins and 61 down-regulated proteins). The protein network was constructed and analyzed using the STRING database and Cytoscape

Table 2 The List of 10 top hub proteins with the highest degrees

The human-readable label name	Description	Uniprot	Degree	Betweenness Centrality
ALB	Serum albumin	P02768	55	0.1523043
FN1	Fibronectin	P02751	49	0.08806922
VEGFA	Vascular endothelial growth factor-A	P15692	38	0.06025576
FGA	Fibrinogen alpha chain	P02671	35	0.02468968
EGF	Epidermal growth factor	P01133	35	0.06421301
PLG	Plasminogen	P00747	32	0.02962186
FGG	Fibrinogen gamma chain	P02679	31	0.01531035
KNG1	Kininogen-1	P01042	31	0.03993729
P4HB	Protein disulfide isomerase	P07237	30	0.05458324
APOA1	Apo lipoprotein A-I	P02647	30	0.02361636

Table 3 The PCOS-drug subnetworks. The table represents the shared proteins between MCODE subnetworks and drugs in which the relationships were statistically significant

PCOS subnetwork	Density score	Number of Nodes	Number of Interactions	Associated drugs	Shared proteins
No.2	7.75	17	62	Spironolactone	VEGF-A, TGFB1, AGT
				Pioglitazone	AGT, EGF, VEGFA, TGFB1
				Metformin	AMBP, RBP4, AGT, VEGFA, TGFB1
				Letrozole	VEGF-A, TGFB1
No.3	6.353	18	54	Rosiglitazone	HSPB1, HSPD1, ACO2, TALDO1, VDAC1, MDH2, HSPA5, ATP5J, BCL2L1, PKM

3.5.1 software. The PPI network from the "significantly altered PCOS proteins was then analyzed using the Cytoscape Network Analyzer tool to identify hubs and bottlenecks [34]. Table 2 represents ten top hub proteins with the highest degrees. Figure 2 depicts the PCOS PPI network, and the hub nodes are shown with a bigger size, and their colors are closer to dark red color. The top ten hubs were functionally enriched using GeneMANIA, and the results are shown in Supplementary Fig. S1. Each hub node is depicted with various colors in the supplementary
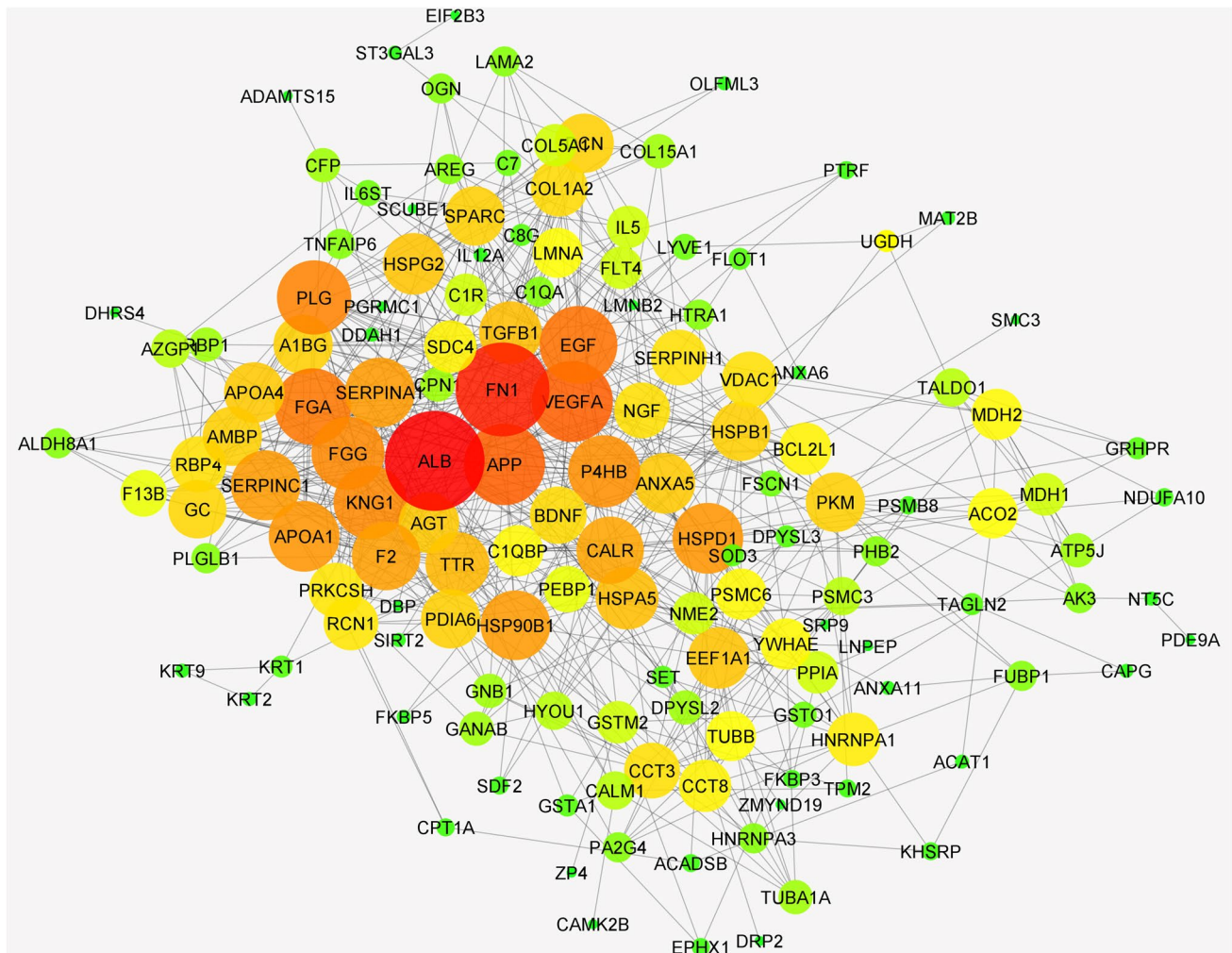
Fig. 2 PCOS PPI network. The PCOS PPI network is visualized using Cytoscape 3.5.1 software. The top ten hubs (nodes with a higher degree) are shown with a bigger size, and their color is closer to dark red

figure, and the different colors represent different biological functions.

PCOS PPI MCODE clusters

The MCODE plugin identified five subnetworks as significant in the PCOS PPI network. (The MCODE parameters settings included Degree Cutoff = 2, Node Score Cutoff = 0.2, K-Core = 2, and Max-Depth = 100((Table S3). Figure 3 depicts the highly connected regions (clusters) identified using the MCODE app.

PCOS-drug subnetwork

After analyzing the data using the chi-squared test or Fisher's exact test (based on prerequisites), we found that five drugs had significant relations with two of the five PPI subnetworks (subnetworks No.2 and No.3, p-value < 0.05). (Supplementary Table S4) These five drugs included metformin, letrozole, pioglitazone, spironolactone, and rosiglitazone. Subnetwork No.2 showed a significant relationship with pioglitazone, metformin, spironolactone, and letrozole. We

found that subnetwork No.3 was significantly associated with rosiglitazone. The results and shared proteins between subnetworks and drugs are available in Table 3 and Table S5.

Three proteins of subnetwork No.2 were found to be shared with spironolactone-related proteins. These included VEGFA (Vascular endothelial growth factor), TGFB1 (Transforming Growth Factor beta-1), and AGT (Angiotensin). PCOS showed association with pioglitazone in subnetwork No.2 with four shared proteins, including VEGFA, TGFB1, AGT, and EGF (*Epidermal growth factor*). Subnetwork No.2 also highlighted the relation of PCOS with metformin. The VEGFA, TGFB1, AGT, AMBP (Alpha-1-Microglobulin/Bikunin Precursor), and RBP4 (Retinol binding *protein* 4) were shared proteins between PCOS subnetwork No.2 and metformin. Besides, letrozole was accompanied by Subnetwork No.2 and shared some proteins, including VEGFA and TGFB1. Interestingly, the results showed that VEGFA and TGFB1 were the essential shared proteins among subnetwork No.2 and all the four drugs' proteins.

Subnetwork No.3 showed a significant relation with rosiglitazone due to ten shared proteins, including HSPB1
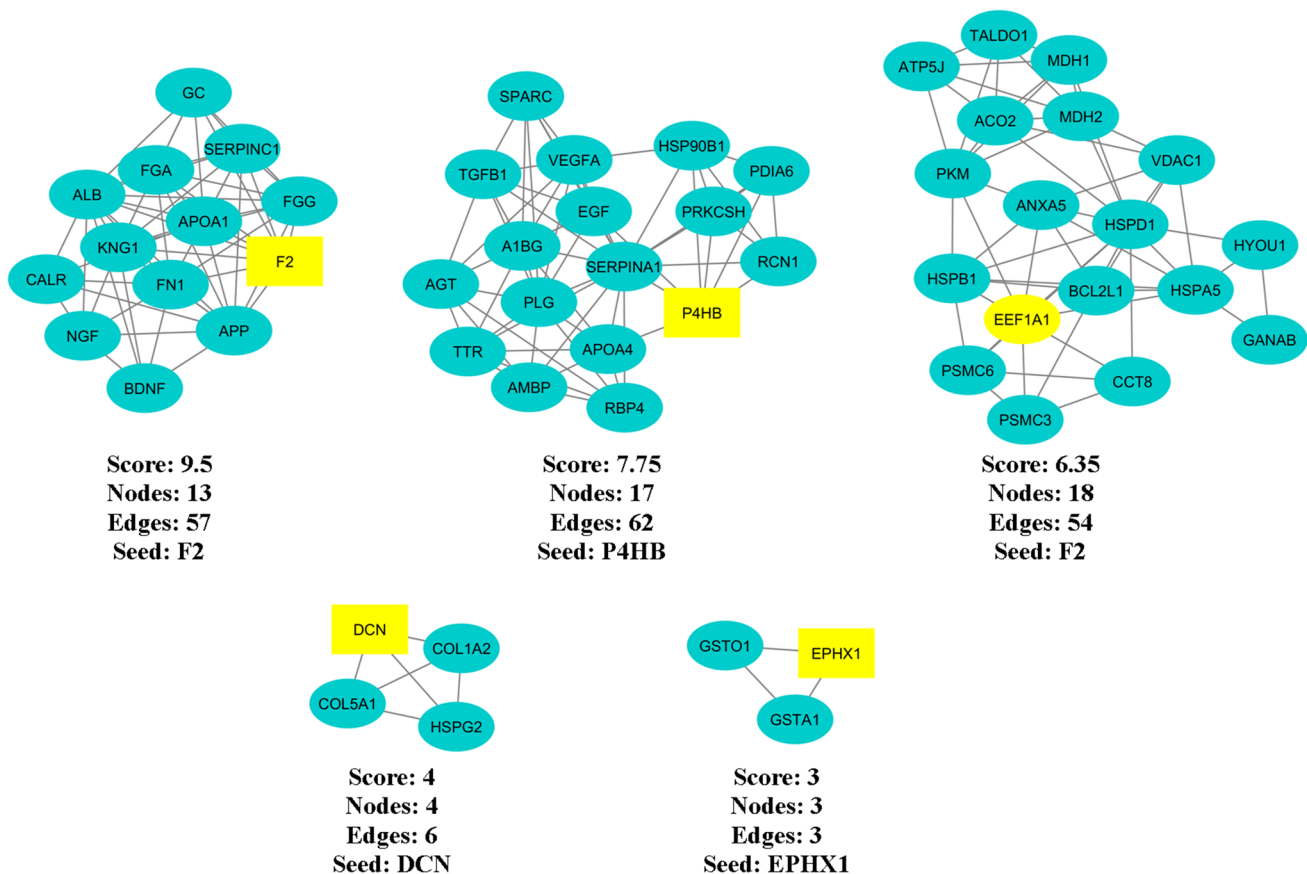

Fig. 3 The figure represents the MCODE clusters. The PPI subnetworks (clusters) are created using the MCODE app from highly connected regions of the PCOS PPI network. The yellow nodes represent the seeds

(Heat Shock Protein Family B (Small) Member 1), HSPD1 (Heat Shock 60 kDa Protein 1), ACO2 (Aconitase 2), TALDO1 (Transaldolase 1), VDAC1 (Voltage-dependent anion-selective channel 1), MDH2 (Malate Dehydrogenase 2), HSPA5 (heat shock 70 kDa protein 5), ATP5J (ATP synthesize-coupling factor 6), BCL2L1 (Bcl-2-like protein 1), and PKM (Pyruvate kinase).

Biochemical pathway and biological process enrichment analysis

The biochemical pathways involved in both PCOS subnetworks (NO.2, NO.3) and the five drugs were analyzed using pathway enrichment analysis by the DAVID tool (Tables S6, S7). The shared pathways between PCOS subnetworks and the drugs were then investigated (Table S8). Interestingly, the PI3K-Akt signaling pathway was a significant pathway between subnetwork No." 2 and the four results of drug-related protein enrichment (pioglitazone, spironolactone, letrozole, and metformin). VEGFA and EGF were found to be the two shared proteins in the PCOS-drug network. They were also both involved in the PI3K-Akt signaling pathway. (Fig. 4).

Six biochemical pathway terms identified as significantly enriched terms in both subnetwork No.3 and rosiglitazone included 1-metabolic pathways, 2-Carbon

Fig. 4 The figure represents the interactions among the four drugs and PCOS subnetwork No.2. The ellipses show the PCOS-related proteins, octagons represent the drugs, and the squares depict pathways. The shared proteins between PCOS subnetwork No.2 and the four drugs (Metformin, Pioglitazone, Letrozole, and Spironolactone) are red. PCOS-drugs interactions are shown by edges in four colors, including red for metformin, orange for spironolactone, green for pioglitazone, and violet for letrozole. The light blue lines indicate the interactions of the PI3K-AKT pathway. Yellow nodes and black edges indicate the PCOS-related proteins' interactions in subnetwork No.2 that are not shared with drug-related proteins

metabolism, 3-citrate cycle (TCA cycle), 4-pyruvate metabolism, 5-biosynthesis of amino acids, and 6-glyoxylate/dicarboxylate metabolism. The shared proteins between subnetwork No.3 and rosiglitazone included: HSPB1, HSPD1, ACO2, TALDO1, VDAC1, MDH2, HSPA5, ATP5J, BCL2L1, and PKM. The shared pathways and proteins between subnetwork No.3 and rosiglitazone are shown in Fig. 5. Table 4 represents the shared pathways significantly enriched for the PCOS-drug subnetworks. The shared biochemical pathways related to the ovaries, encompassing the shared genes, are given in Table 4. (Other results are available in Table S8).

The biological processes intermediating in both subnetworks (NO.2, NO.3) and the five drugs were analyzed using the GO enrichment analysis by the DAVID tool (Tables S9, S10). The results showed that the shared proteins between PCOS subnetworks and drugs participated in various biological processes. They were involved in different vital biological processes, including 1-secretion, 2-response to stress, 3-regulated exocytosis, and 4-platelet degranulation. Table No.5 represents the shared biological processes between enrichment results of the drug-related proteins and PCOS cluster proteins. The top three shared biological process terms between each drug and subnetwork and their shared genes are represented in Table 5. (The other results are available in Table S11; ranked base on *P*-value(
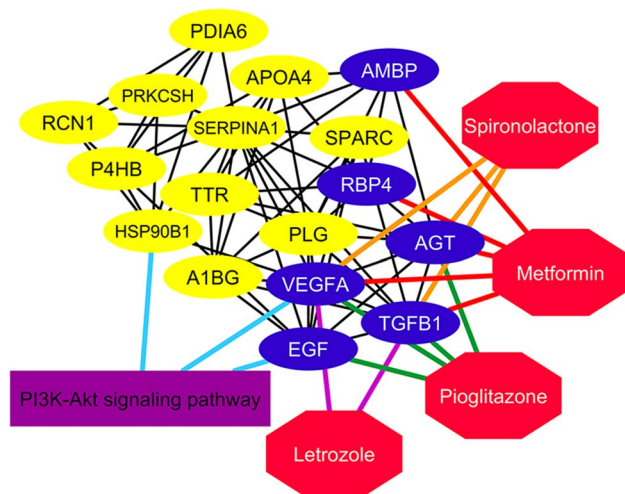
Drug-protein interaction analysis

We evaluated the twenty proteins for their drug-protein interactions with previously US FDA-approved drugs available in DrugBank. DrugBanak is a database that has provided information on protein-drug direct and indirect interactions. Our selected target proteins included the PPI hub-bottlenecks and the shared molecules between PCOS clusters and drug-related proteins (our new repurposed drugs are represented in Table 6). Figure No.6 depicts the drug-protein interactions (direct and indirect) visualized using Cytoscape. For example, copper interacted with eight proteins (PKM, PLG, AGT, APOA1, KNG1, P4HB, HSPD1, HSPA5). Zinc chloride and zinc sulfate had interactions with FN1, P4HB, APOA1, KNG1, FGA proteins. Moreover, zinc and zinc acetate interacted with FN1, P4HB, APOA1, KNG1, FGA, AGT proteins. Reteplase and alteplase were two other drugs interacting with FGA and PLG proteins. VEGF and ALB proteins were related to a medicine named gliclazide. Hyaluronidase (ovine) was linked to ALB and TGFB1. Figure 6 depicted the drug-protein interaction network. Supplementary Table S11 represents the repurposed US FDA-approved medications for PCOS and the identified PCOS crucial proteins.

Fig. 5 The figure shows the interaction**s** between Rosiglitazone and PCOS subnetwork No.3. The ellipses indicate PCOS-related proteins, the octagons show the drugs, and the Squares depict the pathways. The figure highlights the shared proteins in blue and the shared pathways in violet. The pathway protein interactions are shown by six color edges (including 1-red for Pyruvate metabolism, 2-yellow for Biosynthesis of amino acids, 3-violet for TCA cycle, 4-blue for carbon metabolism, 5-pink for metabolic pathways, and 6-orange for Glyoxylate and dicarboxylate metabolism). The yellow nodes and black edges indicate interactions among PCOS-related proteins in subnetwork No.3 that are not shared with the drugs-related proteins
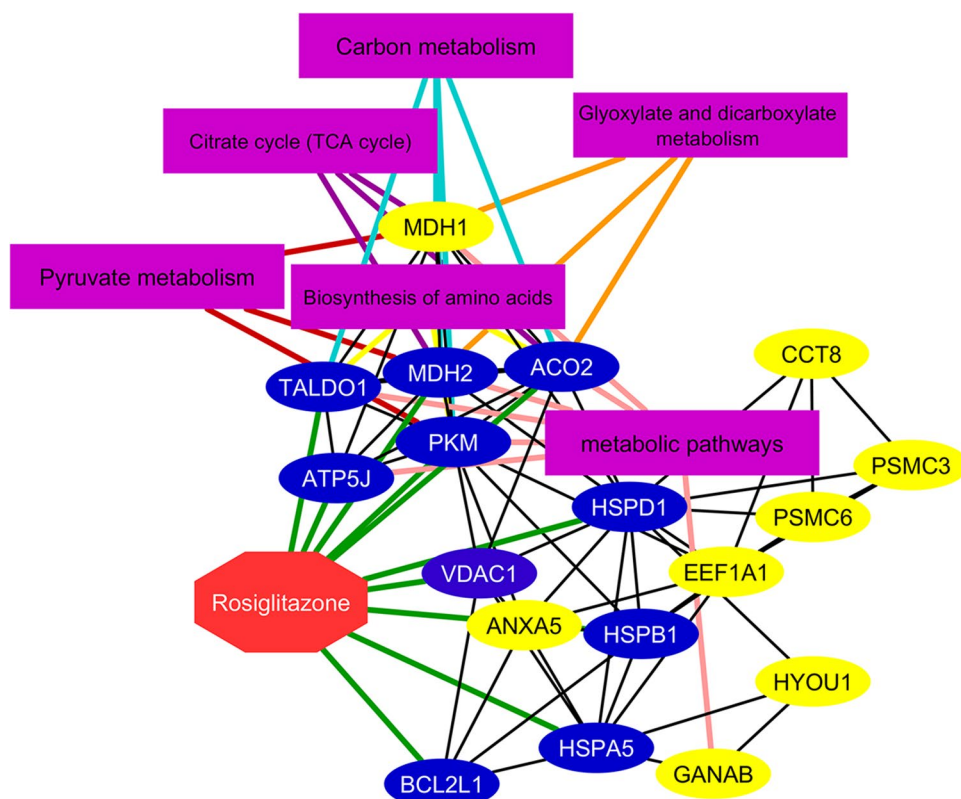

Table 4 PCOS-drug shared pathways. The shared significantly enriched pathways among PCOS, and the drugs were identified using the DAVID web tool

PCOS sub-network	PCOS-associated drugs	Shared pathway
No.2	Spironolactone	PI3K-Akt signaling pathway
	Pioglitazone	
	Metformin	
	Letrozole	
No.3	Rosiglitazone	Metabolic pathways
		Citrate cycle (TCA cycle)
		Carbon metabolism
		Pyruvate metabolism
		Biosynthesis of amino acids
		Glyoxylate and dicarboxylate metabolism

Discussion

Several methods are being used to repurpose new potential drugs for the disease. Some of them are drug-based strategies, while others use disease-based strategies. Drug-based strategies use data related to molecular, chemical, pharmaceutical, and genomic information for predicting new therapeutic potentials for existing drugs [35]. However, disease-based strategies depend on phenotypic traits information, indication information, and side effects to predict therapeutic potentials for existing drugs [36]. Computational drug repositioning methods apply machine learning, network analysis, bipartile graph, clustering, and network centrality measures [37]. This study used a drug-based strategy and applied the proteomics data, network analysis, and cluster identification to predict other potential medications for PCOS treatment. We analyzed hub and bottleneck proteins in the PCOS network. Besides, we investigated the relationship between several drugs and the PCOS MCODE clusters.

Here we show that some nodes with the highest degree values (hub) play essential roles in PCOS patients' pathophysiology. (Table 2) Serum albumin (ALB) had the highest degree and was considered as a hub. According to one previous study, Serum albumin (ALB) was down-regulated in the ovaries of PCOS patients [38]. Albumin is a globular protein that binds to various bioactive molecules, including water, $Ca2+$, $Na+$, $K+$, fatty acids, and hormones [39]. In Buffalo, albumin enhances the maturation and fertilization rate of oocytes and improves the grade of COCs [40]. Fibronectins (FN1, FGA, and FGG) were another group of proteins with a high degree, which were down-regulated in PCOS patients' follicular fluid. They are implicated in integrin-mediated cell adhesion, assembly of the extracellular matrix, and multi-modular protein structures [41]. It

Table 5 PCOS-drug shared biological processes. The shared significantly enriched biological processes between PCOS and the drugs were identified using the DAVID web tool

Subnetwork number	Drugs	Biological process	P-value	Shared proteins	GO ID
No.2	Spironolactone	secretion	2.61E-06	AGT, VEGFA, TGFB1	GO:0,046,903
		single-organism transport	3.81E-05	AGT, VEGFA, TGFB1	GO:0,044,765
		single-organism localization	6.65E-05	AGT, VEGFA, TGFB1	GO:1,902,578
No.2	Pioglitazone	platelet degranulation	4.42E-10	VEGFA, TGFB1, EGF	GO:0,002,576
		regulated exocytosis	1.67E-07	VEGFA, TGFB1, EGF	GO:0,045,055
		secretion by cell	9.91E-07	VEGFA, TGFB1, EGF, AGT	GO:0,032,940
No.2	Metformin	negative regulation of the cellular process	3.56E-04	AMBP, RBP4, AGT, VEGFA, TGFB1	GO:0,048,523
		response to stress	5.37E-04	AMBP, AGT, VEGFA, TGFB1	GO:0,006,950
		negative regulation of the biological process	7.04E-04	AMBP, RBP4, AGT, VEGFA, TGFB1	GO:0,048,519
No.2	Letrozole	tube development	1.54E-04	VEGFA, TGFB1	GO:0,035,295
		response to stress	5.37E-04	VEGFA, TGFB1	GO:0,006,950
		response to oxygen-containing compound	0.001629	TGFB1	GO:1,901,700
No.3	Rosiglitazone	response to organic substance	7.02E-04	PKM, HSPB1, HSPD1, BCL2L1, HSPA5	GO:0,010,033
		organonitrogen compound metabolic process	7.39E-04	PKM, HSPB1, MDH2, ATP5J, MDH1	GO:1,901,564
		small-molecule metabolic process	0.001909	PKM, TALDO1, ACO2, MDH2, ATP5J, MDH1	GO:0,044,281

Fig. 6 Drug-protein interactions. Green nodes depict the proteins, and pink nodes represent the drugs. The dashed lines (-–– -) and the continuous lines (_____) show direct and indirect interactions, respectively

has been reported that Fibronectin affects cumulus expansion and polar body extrusion [42], so it regulates cell adhesion and plays a role in oocyte maturation. Plasminogen (PLG) and Kininogen-1 were the other two proteins with high degrees that were down-regulated and up-regulated in PCOS patients, respectively [41, 43]. The roles of some hub proteins in the PCOS pathogenesis are already verified. We predict that further experimental studies would probably validate the part of other hubs in PCOS pathogenesis.

We investigated the significance of the network relationships among the seven US FDA-approved drugs and PCOS MCODE clusters in the second section. Five drugs were significantly related to two of the PCOS MCODE motifs based on the Chi-square/Fisher's exact test (p-value < 0.05). The shared proteins between the drug-related proteins and PCOS clusters were identified as essential in PCOS pathogenesis. The PCOS-drug subnetwork No.2 depicts the relationship between PCOS and metformin, letrozole, pioglitazone, and spironolactone. VEGF, EGF, TGFB1, AGT, AMBP, RBP4 proteins, and the PI3K/AKT pathway were shared (Fig. 4). VEGF and EGF are related to the PI3K/AKT pathway. They have the highest degree among the shared proteins.

The PI3K/PTEN/AKT pathway has fundamental cellular functions including, growth, survival, transcription, translation, and proliferation [44]. It plays crucial regulatory roles in ovarian function, such as activation and survival of primordial follicles. It also affects oocyte maturation and regulates the proliferation and differentiation of granulosa and theca cells. In 2014, Makker et al. showed that irregularities in the PI3K pathway associates with impaired follicular/oocyte development and impaired ovulation [45]. Besides, in 2019, Tian-Yu Zhang et al. showed that exposure to Ochratoxin A impairs the proliferation and apoptosis of Granulosa cells through the PI3K/AKT pathway in a porcine model [46].

VEGF was found as a shared protein among subnetwork No.2 and four drugs (Metformin, Letrozole, Pioglitazone, and Spironolactone). VEGF acts as an angiogenesis factor. VEGF increases in granulosa, theca, and luteal cells of PCOS patients. It also rises in their follicular fluid. Increased VEGF leads to increased blood flow, vascularization, and Ovarian Hyperstimulation Syndrome (OHSS) in PCOS patients [47]. The PI3K/AKT pathway leads to an increase in angiogenesis through an increase in VEGF. Tiazolididone drugs (TZD), including rosiglitazone, pioglitazone, and troglitazone, have been reported to inhibit VEGF-induced angiogenesis [48]. Other drugs, such as metformin, spironolactone, and letrozole, also have anti-angiogenic effects. They reduce the risk of ovarian hyperstimulation syndrome (OHSS) by lowering the VEGF level [49–51].

The other shared protein was EGF. In PCOS patients, the epidermal growth factor (EGF) increases [52]. In granulose cells, EGF inhibits estrogen synthesis and blocks

antral follicle growth. It leads to follicular arrest in PCOS patients [53]. The EGF increment in women with POCS can lead to disorders in ovarian function through PI3K/AKT/mTORC1. So, EGF, a shared protein between subnetwork No.2 and pioglitazone, is involved in the PI3K/AKT pathway. Takata Y et al. reported the pioglitazone as a specific antagonist of EGF that inhibits the EGF receptor tyrosine kinase [54]. So, pioglitazone probably reduces the activation of EGF through the inhibition of its target receptor.

TGFB was another shared protein between subnetwork No.2 and metformin, letrozole, pioglitazone, and spironolactone. This protein probably inhibits meiotic resumption in porcine cumulus-oocyte complexes [55]. Letrozole inhibits estrogen production by repressing the aromatase enzyme [56]. Meimei Liu et al. reported that metformin reduces TGF-B1 and improves chronic inflammation [57]. Yamada et al. suggested that pioglitazone can suppress the TGF-B superfamily and improve these patients [58]. Spironolactone is an anti-androgen drug that can reduce androgens and decrease TGF-B [59].

Angiotensin (AGT), the other shared protein between subnetwork No.2 and Metformin, Pioglitazone, and Spironolactone, is down-regulated in follicular fluid of PCOS patients [41]. Angiotensin II receptors are located on steroidogenic cells and are involved in synthesizing steroid hormones [60, 61]. The steroid hormones are considered as possible markers for oocyte maturation and cumulus expansion [62]. Wei Zhang et al. showed that pioglitazone increases the expression of Angiotensin-Converting Enzyme 2 (ACE2) [63]. Spironolactone is an antagonist for aldosterone and androgen receptors. It can be used in the treatment of PCOS [64]. The α-1-microglobulin/bikunin precursor (AMBP), the other shared protein between subnetwork No.2 and metformin, is a component of the inter-α-trypsin inhibitor chain and acts in the Cumulus Oocyte Complex (COC) matrix formation and its expansion [65]. Down-regulation of AMBP and TNFAIP6 (TNF Alpha Induced Protein 6) disrupts the matrix organization and expansion of the COCs in PCOS [66].

Retinol-binding protein 4 (RBP4), the shared protein between subnetwork No.2 and metformin, is expressed in theca cells. High levels of this protein were observed in fluids of follicular cysts [67]. The RBP4 level is also higher in fluids of large follicles in comparison with fluids of small follicles. RBP4 acts in retinol transport and accumulation in follicular fluids of the dominant follicles [68]. It is involved in retinoid homeostasis and the physiological function of the ovaries. Regulation of RBP4 expression during follicle development is essential for altering the ovary's retinoid levels during follicle development [69]. These correlations suggest that AGT, AMBP, and RBP4 are affected by metformin, but the exact mechanisms by which metformin affects them

appear to be unknown. Discovering the action mechanism of metformin and these proteins may also better explain these proteins' role in the disease phenotype.

Rosiglitazone was significantly related to PCOS subnetwork No.3. Their shared proteins included HSPB1, HSPD1, ACO2, TALDO1, VDAC1, MDH2, HSPA5, ATP5J, BCL2L1, and PKM. Pyruvate metabolism, Biosynthesis of amino acids, Tricarboxylic acid cycle (Krebs cycle), Carbon metabolism, Glyoxylate, Dicarboxylate metabolism, and Metabolic pathways were the other significant shared biochemical pathway terms between PCOS and rosiglitazone. These findings suggest that improving the phenotype of the disease in PCOS patients by rosiglitazone is also likely by regulating metabolic pathways.

Zinc and copper were identified as two repurposed medications. Interestingly, in women with PCOS syndrome, zinc levels are reduced. The zinc level is introduced as one of the possible causes of insulin resistance in these patients. The use of zinc compounds can improve their insulin resistance and lipid metabolism [70]. Some antioxidant enzymes require copper and zinc for having a proper function in oocyte maturation, ovulation, and fertilization [71]. Zinc plays a role in homeostasis, cell growth, hormone release, immunological responses, and biological reproduction. It also protects cells against reactive oxygen species [72].

Reteplase and alteplase were two other medications interacting with FGA and PLG. They are novel recombinant plasminogen activators that cleavage the Arg-Val bound of endogenous plasminogen to generate plasmin [73, 74]. An increase of plasmin in the ovary can inhibit follicular rupture and improve ovulation [75]. Reteplase affects the lytic system and is an agent that contributes to the moderating of the lytic system by increasing some specific Fibrins. [76]. Gliclazide was another repurposed drug affecting VEGF and ALB. Gliclazide inhibits neovascularization through the down-regulation of VEGF. It also can suppress oxidative stress [77]. Therefore, gliclazide can be recommended as a novel therapeutic strategy in the treatment pathophysiology of PCOS. Hyaluronidase was also another identified drug that interacted with ALB and TGFB1 proteins. Hyaluronidase is reported to disrupt the extracellular matrix of oocytes and improve embryo development [78]. Previous studies have shown that removing oocyte cumulus cells (before inoculation) increases oocyte fertilization potential [79].

This study constructed and topologically analyzed the PCOS protein–protein interaction network and identified PCOS network hub/bottleneck proteins. Besides, we identified the critical proteins interacting with drugs currently used to improve ovulation in PCOS patients based on the significant relations between PCOS clusters and drug-related proteins using Fischer exact test analysis (AGT, PKM, HSPD1, HSPA5, P4HB, KNG1, APOA1, PLG, FN1, FGA, FGG, VEGF, ALB, TGFB1, BCL2L1, PKM, MDH2,

VDAC1, TALDO1, RBP4, EGF). We attempted to predict new medications targeting the critical or similar identified proteins using the DrugBank database. Hao Huang et al. also reported a new computational approach to determine PPDT-Modules and PCOS potential drug targets in protein–protein interaction networks (PPIN). In their study, one PPDT-Module and 21 PCOS drug targets were identified, which 42 drugs targeting 13 PCOS drug targets (ESR1, RXRA, NCOA1, ESR2, THRB, RARA, PPARA, PPARG, PGR, ESRRG, RXRB, RARG, and VDR) were reported to be previously investigated experimentally [80]. Yu Wang et al. also attempted to predict candidate target proteins related to PCOS and its known targets for clinical drugs and suggested some potential candidate targets, including ESR1, PGR, AR, AKR1C3, INSR, THRB, PTPN1, DPP4, NR3C1, HSD11B1, and METAP2 for berberine and other drugs related to PCOS. They also constructed a drug-target network and analyzed it [81].

In brief, some results of this in-silico analysis in identifying the crucial molecular mechanisms underlying the PCOS pathogenesis have been verified by other previous experimental studies. Some other findings within this text-mined in-silico prediction demand further study in vitro and in vivo. In this study, we also repurposed some new drug candidates for PCOS treatment (Table 6). Herein, we discuss that some of our drug candidates have already shown beneficial effects in improving PCOS patients' symptoms. Therefore, we recommend other repurposed drugs to be experimentally investigated in vitro and in vivo for their possible healing effect in PCOS.

Conclusions

In this study, the significant relationships between a PPI MCODE cluster and most investigated drugs revealed the pivotal role of the PI3K/AKT pathway in PCOS pathogenesis. The PI3K/AKT pathway probably intermediates PCOS pathogenesis through cell proliferation, survival, growth, metabolism, and angiogenesis. Therefore, it may contribute to the PCOS pathogenesis in ovulation and oocyte maturation through the survival of primordial follicles, proliferation/differentiation of granulosa/theca cells, and oocyte maintenance/activation. Our study also predicted some other probable biological processes and pathways underlying PCOS, including the metabolic pathways and the Cell death pathway. Various biological processes were involved in the PCOS pathogenesis, including 1-secretion, 2-response to stress, 3-regulated exocytosis, and 4-platelet degranulation. These probably play vital roles in PCOS molecular pathogenesis too. Some of our in-silico prediction results were recently verified by other experimental studies, while others remain to be further

Table 6 The table represents the repurposed FDA-approved medications for PCOS and the identified PCOS crucial proteins. The repurposed medicines are sorted by the number of proteins interacting with them in the constructed drug-protein interaction network

DrugBank ID	repurposed FDA-approved Drugs	Number of proteins interacting with drug	Proteins
DB09130	Copper	8	AGT, PKM, HSPD1, HSPA5, P4HB, KNG1, APOA1, PLG
DB14487	Zinc acetate	6	P4HB, KNG1, FN1, APOA1, AGT, FGA
DB01593	Zinc	6	AGT, APOA1, KNG1, P4HB, FN1, FGA
DB09322	Zinc sulfate	5	P4HB, FN1, FGA, KNG1, APOA1
DB14533	Zinc chloride	5	KNG1, P4HB, FN1, FGA, APOA1
DB11572	Thrombin alfa	2	FGG, FGA
DB00015	Reteplase	2	FGA, PLG
DB00009	Alteplase	2	FGA, PLG
DB01120	Gliclazide	2	VEGF, ALB
DB00070	Hyaluronidase (ovine)	2	ALB, TGFB1

investigated in vitro and in vivo. In PCOS, treatments had better be selected based on specific patient manifestations and be individualized. Personalized medicine requires in-depth knowledge of the various possible molecular pathologies of the disease. Overall, using a systems biology approach could help predict a more in-depth insight into the molecular pathology behind the PCOS mechanism that could influence the future design of prophylactic and therapeutic drugs and fill our knowledge gap toward personalized medicine. In this study, we identified the crucial molecules underlying PCOS pathogenesis using a systems biology approach. We then repurposed some new candidate drugs for PCOS based on the essential molecules. We recommend them to be investigated in vitro and in vivo for their possible role in healing PCOS.

Declarations

Compliance with ethical standards Not applicable.

Conflicts of interest/Competing interests The authors declare that they have no conflict of interest.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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