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SHP2 improves ovarian morphology and steroidogenic function in a rat PCOS model by modulating IRE1α/XBP1/NLRP3-mediated granulosa cell pyroptosis

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Abstract

Objective: The specific role and mechanism of SHP2 in polycystic ovary syndrome (PCOS) remain unclear. This study aimed to evaluate the therapeutic potential of SHP2 and elucidate the signaling pathways through which it modulates granulosa cell fate in PCOS.

Methods: A PCOS rat model was established via letrozole administration. Rats were divided into three groups (n=6 each): Sham, Model, and SHP2-overexpressing (SHP2-OE). The SHP2-OE group received lentiviral injection prior to modeling. Ovarian histopathology was assessed using Hematoxylin and Eosin (HE) staining. Serum sex hormones were measured by ELISA. Western blotting was used to detect the protein expression of the IRE1α/XBP1/NLRP3 and ZEB1/PKP3 signaling pathways in granulosa cells after different stimulations. Flow cytometry was used to detect granulosa cell apoptosis.

Results: In vivo, SHP2 overexpression significantly ameliorated PCOS-induced ovarian damage, characterized by reduced ovarian weight, fewer cystic follicles, and increased corpora lutea. Hormonally, SHP2-OE decreased levels of estradiol, testosterone, and luteinizing hormone, while increasing follicle-stimulating hormone. Mechanistically, in vitro analysis revealed that testosterone treatment inhibited SHP2 phosphorylation and downregulated PKP3, nuclear E2F1, and CyclinB1. In addition to addition, testosterone activated the IRE1α/XBP1/NLRP3 and ZEB1 pathways, upregulating p-IRE1α, XBP-1s, p-SP1, ZEB1, NLRP3, and GSDMD, thereby promoting granulosa cell apoptosis and pyroptosis.

Conclusion: SHP2 alleviates PCOS-related reproductive endocrine abnormalities and ovarian pathological changes by regulating the IRE1α/XBP1/NLRP3 and ZEB1/PKP3 signaling pathways, thereby influencing granulosa cell pyroptosis and proliferation.

Keywords: SHP2; IRE1α/XBP1/NLRP3; ZEB1/PKP3; granulosa cells; PCOS

Introduction

Polycystic ovary syndrome (PCOS) presents with diverse clinical manifestations, including hyperandrogenemia, ovulation disorders, and is often accompanied by insulin resistance (IR), obesity, and cardiovascular diseases, particularly affecting women of reproductive age. Globally, approximately 5% to 18% of women are affected by PCOS, facing issues such as miscarriage, gestational hypertension, preterm birth, and infertility [1]. The etiology of PCOS is multifactorial, involving complex endocrine and metabolic alterations. [2]Within this context, the dysregulation of ovarian granulosa cells, specifically their proliferation and pyroptosis, has been recognized as a significant contributor to the disease's pathophysiology.[3]

Pyroptosis is distinct from apoptosis as it is a lytic, pro-inflammatory form of programmed cell death driven by gasdermin-mediated membrane pore formation and the release of cytokines like IL-1beta. [4, 5]In the ovarian microenvironment, this inflammatory process is particularly damaging. Increased granulosa cell pyroptosis not only reduces hormone biosynthesis in the ovaries but also exacerbates local inflammation.[6] Consequently, the resulting hormone deficiency disrupts the support for follicular oocytes, impairing their growth and development, and ultimately leading to abnormal follicular development [7].

Recent studies indicate that epithelial-mesenchymal transition (EMT) is a critical driver of ovarian fibrosis and follicular arrest in PCOS, and its activation has been confirmed to be closely associated with the pathogenesis of the disease.[8-10] Key mechanisms driving this pathology involve endoplasmic reticulum stress (ERS) and EMT. The IRE1alpha/XBP1 axis connects cellular stress to the NLRP3 inflammasome activation, promoting pyroptosis, while the ZEB1/PKP3 pathway, known to regulate EMT, controls granulosa cell structural integrity and follicular atresia, thereby contributing to PCOS progression[11].

In recent years, Src homology 2 domain-containing phosphatase 2 (SHP2), as

a multifunctional tyrosine phosphatase, has been shown to play a critical role in cell proliferation, differentiation, apoptosis, and immune responses [12]. Importantly, SHP2 is increasingly recognized as a key immunomodulator that regulates inflammatory cytokines and immune cell activation, functions highly relevant to the chronic inflammation observed in PCOS.[13, 14] Abnormal expression of SHP2 is closely associated with the development of various diseases, including cancer, inflammatory diseases, and metabolic disorders. However, the role of SHP2 in PCOS and its specific mechanisms remain insufficiently studied. Existing research suggests that SHP2 may regulate endoplasmic reticulum stress (ERS)-related signaling pathways, such as the IRE1α/XBP1/NLRP3 pathway, and EMT-related signaling pathways, such as the ZEB1/PKP3 pathway, thereby affecting cell apoptosis, pyroptosis, and proliferation [15, 16]. While these signaling pathways are recognized players in PCOS pathology, the specific role of SHP2 in orchestrating these signals, particularly in the context of its immunomodulatory function, requires further validation.

This study aims to explore the effects of SHP2 on the IRE1α/XBP1/NLRP3 and ZEB1/PKP3 signaling pathways and elucidate its mechanism in PCOS. By constructing a PCOS animal model and using lentivirus-mediated SHP2 overexpression, we systematically investigated the effects of SHP2 on ovarian tissue pathology, serum sex hormone levels, granulosa cell apoptosis, and the expression of key signaling pathway proteins. Through this research, we hope to provide new theoretical insights into the molecular mechanisms of PCOS and lay the foundation for developing SHP2-targeted therapeutic strategies.

1 Materials and Methods

1.1 Cells and Experimental Animals

Eighteen 7-week-old female Sprague-Dawley (SD) rats were provided by Henan Sikebais Biotechnology Co., Ltd., with the experimental animal

production license number [SCXK (Yu) 2021-00013]. The rats weighed 180–210 g and were housed in a well-ventilated environment with controlled temperature (25 ± 2)°C, humidity (45%–65%), and a 12 h/12 h light-dark cycle. Human ovarian granulosa cells (KGN cell line) were purchased from Wuhan Procell Life Science & Technology Co., Ltd. (Wuhan, China). The cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS) and 100 U/mL penicillin-streptomycin at 37°C in a humidified 5% CO2 atmosphere in air.

1.2 Main Reagents

Testosterone propionate was purchased from Qinhuangdao Zizhu Pharmaceutical Co., Ltd. The IRE1α/XBP1 inhibitor 3,6-Dimethoxyamphetamine hydrochloride (DMAD) , SHP2 inhibitor PHPS1, and SP1 inhibitor Plicamycin were purchased from MedChemExpress (MCE). SHP2-overexpressing(SHP2-OE), ZEB1-overexpressing(ZEB1-KD), and PKP3-knockdown (PKP3-KD) lentiviruses were purchased from Yeasen Biotechnology (Shanghai) Co., Ltd. Hematoxylin-eosin (HE) staining kit and RIPA lysis buffer were purchased from Beyotime Biotechnology (Shanghai). Antibodies against p-SHP2, p-IRE1α, XBP-1s, p-SP1, ZEB1, PKP3, nuclear E2F1, CyclinB1, NLRP3, GSDMD, and GAPDH were purchased from Wuhan Sanying Biotechnology Co., Ltd. IgG secondary antibodies and ECL hypersensitive luminescent solution were purchased from Beijing Biosynthesis Biotechnology Co., Ltd.

1.3 Bioinformatics Analysis

Based on the PCOS dataset GSE34526 from the GEO database (including 3 normal samples and 7 PCOS samples), background correction, normalization, and multiple probe averaging were performed using the Robust Multi-array Average (RMA) algorithm, and batch effects were eliminated using the ComBat method. Differentially expressed genes (DEGs) were screened using the limma package, with a threshold of $|\log_2 FC| > 2$ and a corrected p-value (Benjamini-Hochberg method) < 0.05. Significant DEGs were subjected to

multi-level functional annotation: Gene Ontology (GO) enrichment analysis (covering cellular components CC, molecular functions MF, and biological processes BP) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed using the ClusterProfiler package to reveal key biological mechanisms[17, 18]. Additionally, Spearman correlation analysis was used to systematically evaluate the regulatory network correlations between targets such as IRE1α/XBP-1/P38/SP1/ZEB1/PKP3/Rb/E2F1/Cyclin/SHP2/BRD4/NLRP3 and core cell cycle genes (CDK1) and pyroptosis key factors (GSDMD). Finally, ggplot2 was used to visualize differential analysis and interaction networks, providing a theoretical framework for the mechanistic hypothesis of PCOS dysfunction.

1.4 Grouping and Treatment

The 18 Sprague-Dawley rats were randomly divided into sham group, Model group, and SHP2-OE group, with 6 rats in each group. Except for the sham group, the other groups were used to establish a PCOS animal model by continuous intragastric administration of letrozole (1 mg/kg) for 21 days. The sham group received an equivalent volume of vehicle via intragastric administration for 21 days, serving as a procedural control. The SHP2 - OE group was also treated with letrozole in the same way as the Model group to induce the PCOS model. Specifically, one week before the start of the letrozole administration (defining the first day of letrozole administration as Day 0), rats in the SHP2 - OE group were injected with SHP2 - OE lentivirus to induce SHP2 overexpression prior to model establishment. For cell transfection, cells in the logarithmic growth phase were seeded into 6-well plates and transfected with the appropriate lentiviral vectors (SHP2-OE or empty vector control). After 24 hours of incubation, the culture medium was replaced. Stable cell lines were selected using Puromycin (2 μg/mL) for 7 days.

1.5 HE Staining

The left ovary were fixed in 10% neutral formaldehyde solution for 18 hours, dehydrated with gradient ethanol, cleared with xylene, embedded in paraffin, and sectioned continuously at 4 μm. HE staining was performed according to the kit instructions, and the morphological structure of the ovary was observed under an optical microscope. The number of mature follicles and corpora lutea in 5 fields of view was counted under a 400× microscope.

1.6 ELISA for Serum Sex Hormone Levels

Blood was collected from the abdominal aorta of rats anesthetized with 3% isoflurane, centrifuged at 2,000 r/min for 10 min to separate serum, and serum levels of E2, T, FSH, and LH were measured by ELISA according to the kit instructions using a microplate reader.

1.7 Cell Culture and Grouping

The human immortalized granulosa-like tumor cell line KGN (Wuhan Procell Life Science & Technology Co., Ltd. China) was used in this study. As an immortalized cell line derived from a granulosa cell tumor, KGN cells retain the physiological characteristics of primary granulosa cells.The cells were cultured in DMEM/F12 medium supplemented with 10% fetal bovine serum at 37°C in a humidified atmosphere containing 5% CO2. For experiments, cells in the logarithmic growth phase were seeded and allowed to adhere for 24 h prior to any treatment to ensure optimal cellular status. To establish the in vitro model and investigate the underlying mechanisms, cells were divided into the following groups based on the specific treatment conditions. All treatments were applied to granulosa cells as detailed below:

NC (Normal Control) group: Cells were cultured under normal conditions without any intervention.

Testosterone group:Cells were stimulated with 5 μM testosterone propionate (Te) for 24 h to induce the PCOS-like phenotype. [3].

Te + DMAD group:Cells were pretreated with 5 μM DMAD[19], an IRE1α/XBP1 inhibitor, for 24 h, followed by stimulation with Te (5 μM, 24 h)

Te + DMAD + PHPS1 group:Cells were pretreated with 10 μM PHPS1[20],

an SHP2 inhibitor, for 48 h, and DMAD (5 μM, 24 h), followed by Te stimulation (5 μM, 24 h).

Te + DMAD + PHPS1 + Plicamycin group:Cells were pretreated with 200 nM Plicamycin[21], an SP1 inhibitor, for 48 h, along with PHPS1 (10 μM) and DMAD (5 μM), followed by Te stimulation (5 μM, 24 h).

Te + DMAD + PHPS1 + Plicamycin + ZEB1-KD group:Cells were transfected with ZEB1-KD lentivirus prior to drug administration. Subsequently, cells were subjected to the pretreatment regimen (Plicamycin, PHPS1, and DMAD) followed by Te stimulation as described above.

Te + DMAD + PHPS1 + Plicamycin + ZEB1-KD + PKP3-KD group:Cells were co-transfected with PKP3-KD lentivirus and ZEB1-KD lentivirus. After transfection, cells received the same combined drug treatments (Plicamycin, PHPS1, and DMAD) followed by Te stimulation.

1.8 Western Blot

Western blot was used to detect the expression of p-SHP2, p-IRE1α, XBP-1s, p-SP1, ZEB1, PKP3, nuclear E2F1, CyclinB1, NLRP3, and GSDMD proteins in ovarian granulosa cells. Granulosa cells from each group were lysed with RIPA protein lysis buffer on ice, centrifuged for 15 min, and the supernatant was collected. Protein concentration was measured by BCA assay and adjusted to 2 μg/μL as the loading sample. A 10 μL loading sample was subjected to SDS-PAGE electrophoresis and transferred to a membrane. After blocking for 1 h, primary antibodies (1:2,000) and internal reference antibody GAPDH (1:10,000) were added and incubated overnight at 4°C. After washing, secondary antibodies (1:10,000) were added and incubated at 37°C for 1 h. After washing, ECL was added for development, and the images were captured and analyzed for relative protein expression.

1.9 Flow Cytometry for Cell Cycle Analysis

Granulosa cell suspension (5.0×10^8/L) was seeded in a 6-well plate with 2 mL RPMI1640 complete medium and cultured for 24 h. The medium was aspirated, and the cells were cultured according to section 1.7. The cells

were collected, fixed with 70% ethanol, and stored overnight at 4°C. The cells were then incubated with PI in the dark for 15 min, and the cell cycle changes were measured using a flow cytometer. The experiment was repeated 3 times, and the mean value was taken. Flow cytometry data were acquired on a Guava® easyCyte™ (Luminex, USA) flow cytometer using a 488 nm laser for excitation and a 583/26 nm bandpass filter for detection.

1.10 Flow Cytometry for Cell Apoptosis

Annexin V/Alexa Fluor 647 apoptosis kit was used to detect cell apoptosis. Granulosa cells were seeded in a 6-well plate at 5×10^5 cells/well, grouped, and treated as described above. The cells were collected, centrifuged at 1,200 r/min for 5 min, and resuspended in 1× binding buffer to a density of $1–5 \times 10^6$ cells/mL. A 5 µL Annexin V/Alexa Fluor 647 reagent was added, mixed, and incubated at room temperature in the dark for 5 min. A 5 µL PI solution (20 µg/mL) and 400 µL PBS were added, and the samples were immediately analyzed by flow cytometry.

1.11 Statistical Analysis

SPSS 20.0 software was used for statistical analysis of the experimental data. Normally distributed measurement data were expressed as mean ± standard deviation. One-way ANOVA was used for comparisons among multiple groups. LSD-t test was used for pairwise comparisons when the variance was homogeneous, and Dunnett’s T3 test was used when the variance was heterogeneous.All methods used in this experiment were performed in accordance with relevant guidelines and regulations, and conformed to the RULES OF 3R and ARRIVE guidelines.

2 Results

2.1 Bioinformatics Analysis Results

During the data preprocessing stage, boxplots were used to evaluate the standardization effect(Figure 1A), and principal component analysis (PCA) was employed to verify the batch effect correction(Figure 1B). Using the limma package (threshold $|\log_2 FC| > 2$ and adjusted p-value < 0.05), 508

significantly differentially expressed genes (DEGs) were identified, including 377 upregulated and 131 downregulated genes, with the inflammasome component NLRP3 significantly upregulated(Figure 1C-D). GO/KEGG enrichment analysis based on ClusterProfiler revealed that at the cellular component level, DEGs were significantly enriched in the secretory granule lumen, cell-substrate junction, and tertiary granule membrane(Figure 1Ea); at the molecular function level, they were primarily enriched in immune receptor activity, cytokine binding, and cytokine receptor activity(Figure 1Eb); at the biological process level, key pathways included positive regulation of cytokine production, ROS metabolic process, and superoxide anion generation(Figure 1Ec). KEGG analysis further revealed significant enrichment of DEGs in Chemical carcinogenesis - reactive oxygen species, NOD-like receptor signaling pathway, and FoxO signaling pathway. (Figure 1Fa-Fb)Spearman correlation analysis unveiled the cascade regulatory relationships of core molecules (Figure 1G): the IRE1α-XBP1-P38 axis showed positive synergy, with P38 activating SP1 to drive the expression of the epithelial-mesenchymal transition regulator ZEB1; ZEB1 negatively regulated the desmosomal protein PKP3. In the cell cycle regulation module, PKP3 alleviated the transcriptional repression of E2F1 by Rb protein (Rb-E2F1 negative correlation), thereby activating the E2F1-Cyclin/CDK1 proliferation axis (E2F1-Cyclin positive correlation; E2F1-CDK1 positive correlation) (Figure 1H). Additionally, SHP2 was found to negatively regulate P38, while the epigenetic regulator BRD4 was positively correlated with the IRE1α/XBP1 inflammatory axis (BRD4-IRE1α positive correlation; BRD4-XBP1 positive correlation) and P38. Notably, NLRP3 showed a significant positive correlation with the pyroptosis execution protein GSDMD(Figure 1I), suggesting that inflammasome activation directly drives the pyroptosis process in PCOS granulosa cells.

2.2 SHP2 may improve abnormal steroidogenesis in a PCOS model

The pathological morphology of ovarian tissues worsened (Fig2A), with a

significant increase in the number of cystic dilated follicles and a significant decrease in the number of corpora lutea (Fig2B) in the ovarian tissue. Compared to the sham group, the Model group showed increased levels of estradiol (E2), testosterone (T), and luteinizing hormone (LH), decreased levels of follicle-stimulating hormone (FSH), and increased ovarian weight (Fig2C). Compared to the Model group, the SHP2-OE group showed decreased levels of E2, T, and LH, increased levels of FSH, and reduced ovarian weight. The pathological morphology of ovarian tissues significantly improved, with a significant decrease in the number of cystic dilated follicles and a significant increase in the number of corpora lutea in the ovarian tissue.

2.3 SHP2 Alleviates PCOS Phenotypes via IRE1α/XBP1/NLRP3 and ZEB1/PKP3-Mediated Regulation of Cell Pyroptosis and Proliferation

We first examined the expression of key signaling upstream regulators. In comparison to the NC group, the Te group exhibited a significant decrease in p-SHP2 and PKP3 levels, accompanied by increases in p-IRE1α, XBP-1s, p-SP1, and ZEB1 (Fig. 3). Treatment with the SHP2 activator DMAD (Te+DMAD group) reversed these trends, elevating p-SHP2 and PKP3 while suppressing p-IRE1α, XBP-1s, p-SP1, and ZEB1 (Fig. 3). Conversely, when the SHP2 inhibitor PHPS1 was added to the Te+DMAD group, the expression of p-SHP2 and PKP3 was reduced again, while p-SP1 and ZEB1 levels were upregulated. Notably, p-IRE1α and XBP-1s levels in this group did not differ significantly from those in the Te+DMAD group, suggesting SHP2 regulates specific branches of this pathway (Fig. 3). Further inhibition with Plicamycin (Te+DMAD+PHPS1+Plicamycin group) did not alter p-SHP2, p-IRE1α, or XBP-1s levels, but successfully restored PKP3 expression and reduced ZEB1, p-SP1 levels (Fig. 3). Subsequent introduction of ZEB1-KD (Te+DMAD+PHPS1+Plicamycin+ZEB1-KD group) maintained p-SHP2, p-IRE1α, XBP-1s, and p-SP1 levels at the previous stage, but significantly decreased ZEB1 and increased PKP3 (Fig. 3). Finally, in the

Te+DMAD+PHPS1+Plicamycin+ZEB1-KD+PKP3-KD group, p-SHP2, p-IRE1α, XBP-1s, p-SP1, and ZEB1 levels remained consistent with the previous group, while PKP3 expression was effectively knocked down (Fig. 3).

To determine the functional consequences of these signaling changes, we assessed cell cycle regulators, pyroptosis markers, and cellular phenotypes. Initially, the Te group showed decreased nuclear E2F1 and CyclinB1, but increased NLRP3 and GSDMD (Fig. 4A). These molecular changes corresponded to a reduced G2 phase population and increased granulosa cell apoptosis (Fig. 4B and 4C). Administration of DMAD restored nuclear E2F1 and CyclinB1 levels and reduced NLRP3 and GSDMD, which was accompanied by an increased G2 phase and reduced apoptosis. In the Te+DMAD+PHPS1 group, the suppression of SHP2 led to a decrease in nuclear E2F1 and CyclinB1 and a resurgence of NLRP3 and GSDMD, resulting in a reduced G2 phase and elevated apoptosis. The addition of Plicamycin (Te+DMAD+PHPS1+Plicamycin group) rescued these defects, increasing nuclear E2F1 and CyclinB1 and decreasing NLRP3 and GSDMD, thereby improving the G2 phase and reducing apoptosis. The beneficial effects were further enhanced by ZEB1-KD (Te+DMAD+PHPS1+Plicamycin+ZEB1-KD group), which showed further increases in nuclear E2F1, CyclinB1, NLRP3, and GSDMD, correlating with continued improvements in the G2 phase and apoptosis reduction. However, the final knockdown of PKP3 (Te+DMAD+PHPS1+Plicamycin+ZEB1-KD+PKP3-KD group) reversed these protective outcomes, evidenced by significantly decreased nuclear E2F1, CyclinB1, NLRP3, and GSDMD, a reduced G2 phase, and a decrease in apoptosis (Fig. 4).

3 Discussion

PCOS is a complex endocrine and metabolic disorder, and its pathogenesis

involves abnormal regulation of multiple molecular signaling pathways [22]. While its etiology is multifactorial, emerging evidence highlights the critical role of intracellular signal transduction defects in granulosa cell dysfunction. The present study elucidates a novel mechanism whereby the suppression of SHP2 regulates PCOS pathology through the concurrent activation of the IRE1α/XBP1/NLRP3 axis and the p38 MAPK/ZEB1/PKP3 pathway.

Our results demonstrate that testosterone exposure triggers ERS in granulosa cells, evidenced by the activation of the IRE1α/XBP1 pathway. This is consistent with previous reports linking hyperandrogenemia to ERS in PCOS [6, 23]. The endoplasmic reticulum is a crucial site for protein synthesis, folding, and modification within cells. When endoplasmic reticulum function is disrupted, unfolded or misfolded proteins accumulate in the endoplasmic reticulum, triggering ERS. The major effectors of ERS include IRE1α, PERK, and ATF6, with the IRE1α/XBP1 pathway playing a key role in regulating cellular stress responses and inflammatory processes [24-26].

In this context, we observed that activated IRE1α/XBP1 signaling facilitates the phosphorylation of p38 MAPK. Furthermore, our data suggest a positive feedback loop involving p38-mediated phosphorylation of SP1 and subsequent BRD4 transcription, which sustains IRE1α/XBP1 activity[27]. This persistent activation of the stress response represents a key molecular shift in PCOS granulosa cells.

Downstream of these events, phosphorylated SP1 promotes the transcription of ZEB1. ZEB1 is traditionally recognized as a regulator of EMT; however, our findings align with emerging evidence suggesting its suppressive role on PKP3 promoter activity [28], which compromises cell adhesion. PKP3 has been implicated in regulating cell cycle progression via the Rb/E2F1 axis [29]. Specifically, PKP3 inhibits Rb, thereby modulating E2F1 transcriptional activity. In the present study, the reduction in PKP3 correlated with dysregulated E2F1 and CyclinB1 expression, suggesting that the ZEB1/PKP3

axis contributes to the abnormal proliferative capacity of granulosa cells observed in PCOS. Interestingly, while E2F1 typically drives cell cycle entry, the altered nuclear E2F1 levels observed here likely reflect a broader disruption of cell cycle checkpoint controls consistent with PCOS pathology.

Beyond the observed proliferative deficits, this study elucidates a critical mechanism whereby ERS-induced oxidative stress modulates SHP2 to orchestrate granulosa cell fate in PCOS. Our data demonstrate that the accumulation of reactive oxygen species (ROS) under conditions of ERS is significantly associated with the inhibition of SHP2, a multifunctional tyrosine phosphatase central to cellular homeostasis[30]. Inhibition of SHP2 led to the activation of p38 MAPK and the NLRP3 inflammasome. Phosphorylation of p38 MAPK not only participates in the activation of SP1 but can also induce granulosa cell pyroptosis by promoting the expression of NLRP3. The NLRP3 inflammasome is a crucial component of the innate immune system, and its activation can lead to the activation of caspase-1 and the release of pro-inflammatory factors such as IL-1β and IL-18, further exacerbating inflammatory responses and cell damage[31]. In PCOS, inhibition of SHP2 promotes granulosa cell pyroptosis through the activation of the p38/NLRP3 pathway, exacerbating ovarian dysfunction. SHP2 inhibits the phosphorylation of p38 MAPK, blocking the activation of SP1 and NLRP3, thereby inhibiting granulosa cell pyroptosis and inflammatory responses[32]. SHP2 also promotes E2F1 activity by regulating the ZEB1/PKP3 pathway, leading to granulosa cell proliferation. Additionally, SHP2 alleviates endoplasmic reticulum stress-induced cell damage by inhibiting the activity of the IRE1α/XBP1 pathway.

In conclusion, this study demonstrates that hyperandrogenism activates the SHP2/IRE1α/XBP1 signaling pathway, driving the ZEB1/PKP3 axis to trigger follicular arrest in PCOS. Our results show that SHP2 overexpression recapitulates PCOS-like features, confirming its role as a critical pathogenic driver. Therefore, we propose that inhibiting SHP2 activity, rather than

broadly targeting it, is a viable therapeutic strategy. This is supported by our findings that the SHP2 inhibitor PHPS1 effectively reversed ER stress and apoptosis, rescuing granulosa cell function. These insights provide important preclinical evidence for the development of specific SHP2 inhibitors as a novel treatment for PCOS.(Fig 5)

Figure Captions:

Figure 1:

A. Boxplot for data standardization evaluation. B. Principal component analysis scatter plot. C. Volcano plot of differentially expressed genes. D. Heatmap of differentially expressed genes. Ea. GO cellular component (CC) enrichment bubble plot. Eb. GO molecular function (MF) enrichment bubble plot. Ec. GO biological process (BP) enrichment bubble plot. Fa. KEGG pathway enrichment bubble plot. Fb. KEGG pathway enrichment lollipop plot. G. Correlation pie chart of key regulatory factors (IRE1α, XBP-1, p38, SP1, ZEB1, PKP3, Rb, E2F1, Cyclin, SHP2, BRD4). H. Scatter plot of E2F1-CDK1 co-regulation. I. Scatter plot of NLRP3-GSDMD pyroptosis axis.

Figure 2:

SHP2 improves PCOS phenotypes.A. HE staining of ovarian tissue pathology. B. Count of cystic dilated follicles and corpora lutea in ovarian tissue. C. ELISA measurement of serum sex hormone levels.*$p < 0.05$, **$p < 0.01$, ns indicates $P > 0.05$; data are represented as the mean ± SD

Figure 3:

SHP2 Alleviates PCOS Phenotypes via IRE1α/XBP1/NLRP3 and ZEB1/PKP3-Mediated Regulation. A. Western blot detection of SHP2-regulated IRE1α/XBP1/ZEB1/PKP3 protein pathways. B. Quantitative analysis of protein expression levels..*$p < 0.05$, **$p < 0.01$, ns indicates $P > 0.05$; data are represented as the mean ± SD

Figure 4:

SHP2 affects granulosa cell pyroptosis and proliferation. A. Western blot detection of nuclear E2F1, CyclinB1, NLRP3, and GSDMD protein

expression.Cell cycle (B) and apoptosis (C) detected by flow cytometry.*$p < 0.05$, **$p < 0.01$, ns indicates $P > 0.05$; data are represented as the mean ± SD

Figure 5:

SHP2 improves ovarian morphology and steroidogenic function in a rat PCOS model by regulating the IRE1α/XBP1/NLRP3 and ZEB1/PKP3 signaling pathways, thereby influencing granulosa cell pyroptosis and proliferation.

Author Contributions:

L.L.B wrote the first draft of the manuscript. D.X.W and J.N.W 's main work is data collection and analysis. B.Y and X.Z are responsible for bioinformatics analysis and statistical analysis. X.Y.F was in charge of the experimental part of the manuscript. Q.D and Y.Y.W was in charge of the revision of the manuscript. All authors have reviewed the manuscript.

Ethics approval

This study was approved by the Medical Ethics Committee of the 980th Hospital of the Joint Logistics and Security Force of the Chinese People's Liberation Army. (Approval number: 2022-KY-59).

Patient consent for publication

Not applicable

Conflict of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships.

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None.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Fig2

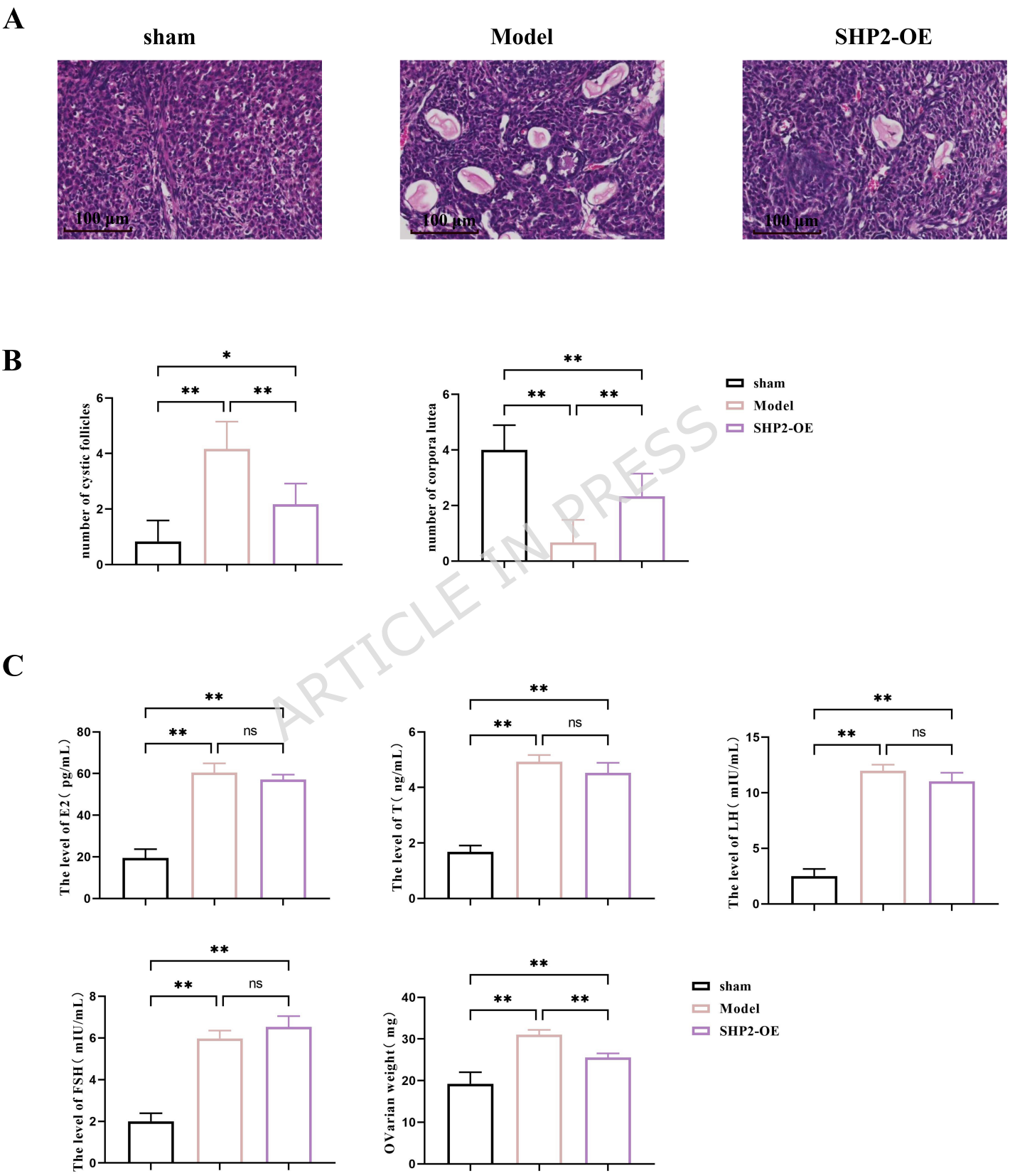

Fig3

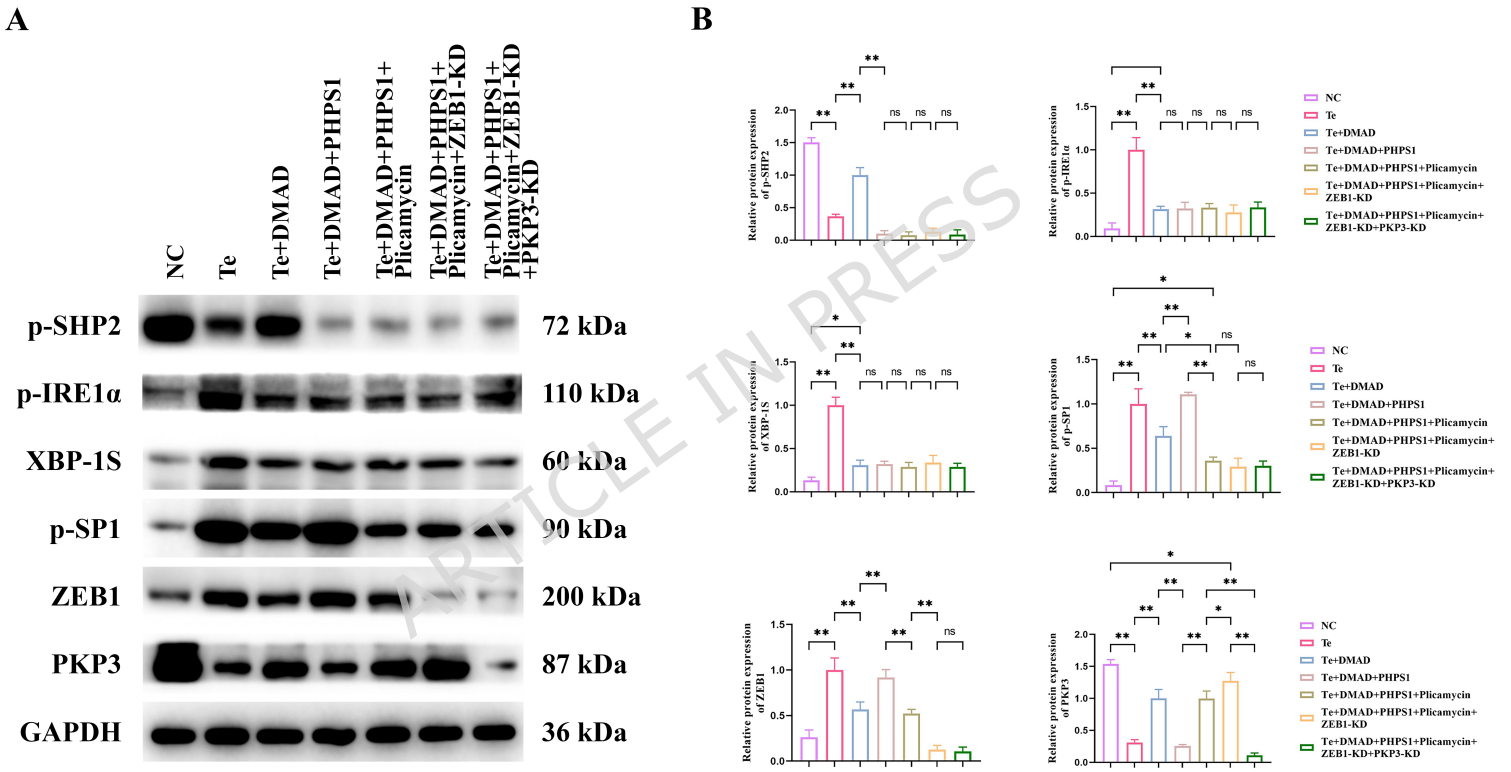

Fig4

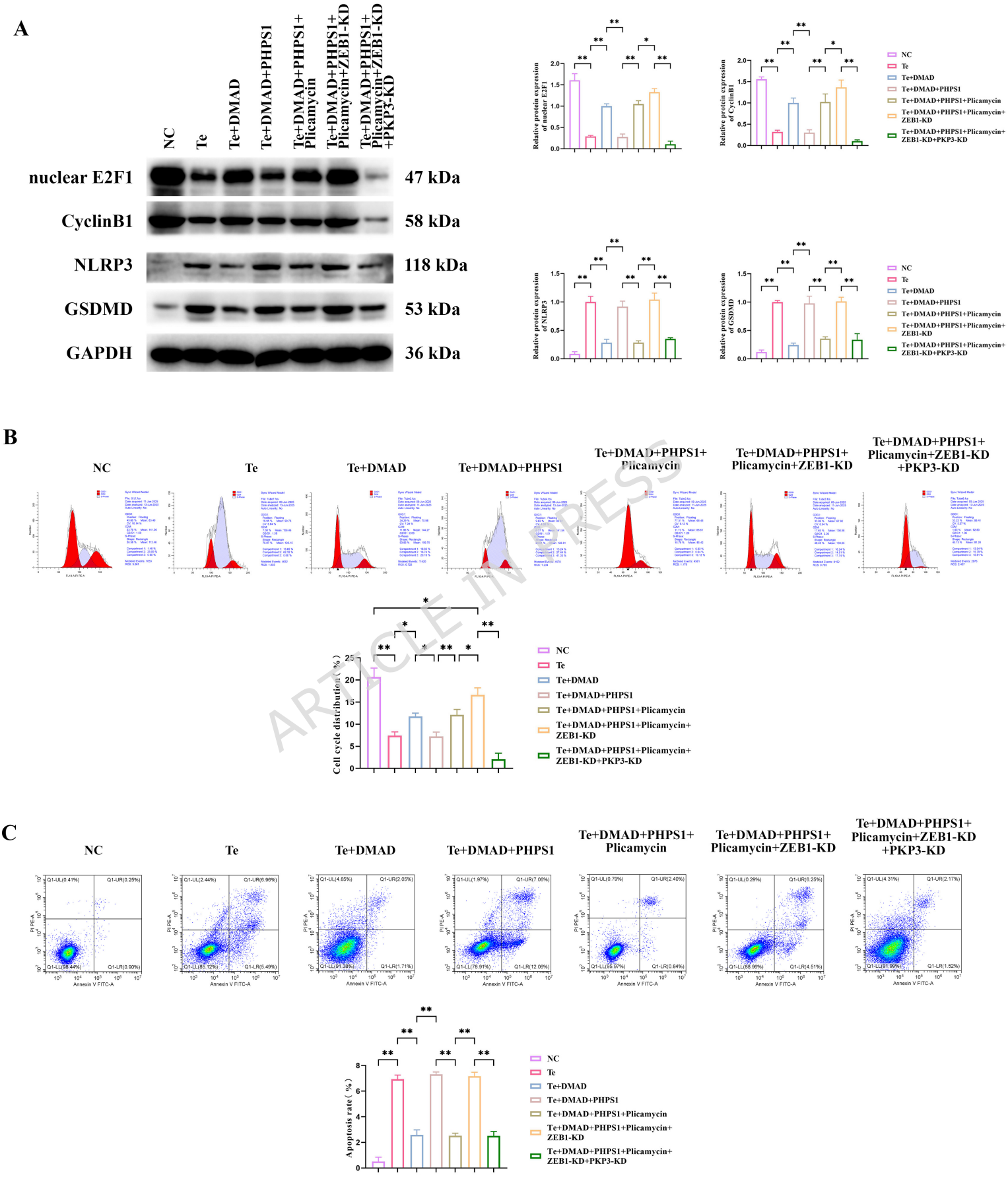

Fig5

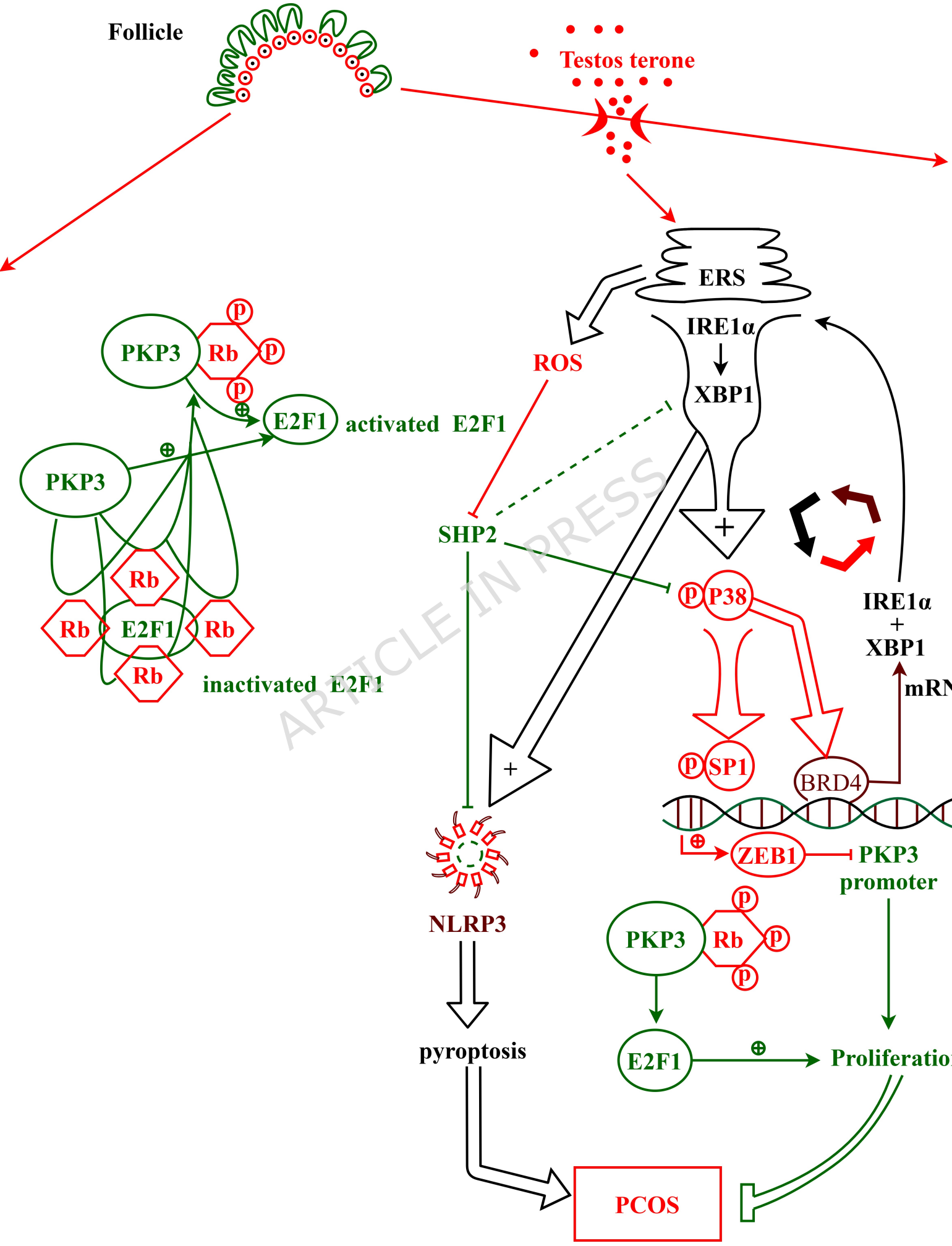

Fig1