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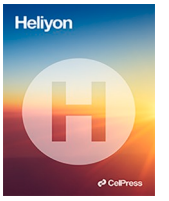

Research article

Investigating the therapeutic mechanism of Jiedu-Quyu-Ziyin Fang on systemic lupus erythematosus through the ERα-miRNA-TLR7 immune axis

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ABSTRACT

Jiedu-Quyu-Ziyin Fang (JQZF) is a formula that has been empirically used for the treatment of SLE in clinical practice. JQZF has become an approved hospital prescription in China. Fifteen MRL/lpr mice were randomly divided into three groups: Model, JQZF, and JQZF + GC, with five mice in each group. Five MRL/MPJ mice were used as the Blank group. After 8 weeks of administration, peripheral blood serum was collected to detect anti-dsDNA antibodies and complement C3 levels. Spleen B cells were collected to detect the expression of TLR7 and NF-κBp65 mRNA, and correlation analysis was performed. Transcriptome sequencing analysis was also performed on spleen B cells. Further, key miRNA and key gene mRNA expression were detected by RT-qPCR, and key protein expression levels were detected by Western blot method. Bioinformatics methods predicted that ESR1 is a key target of JQZF action on SLE, hsa-miR-146a-5p is one of the key miRNAs, and KEGG pathway analysis showed that NF-κB signaling pathway is its key signaling pathway. Transcriptome sequencing of MRL/lpr mouse spleen B cells revealed that the differential genes between the JQZF and Model groups were enriched in Toll-like receptor signaling pathway, NF-κB signaling pathway, Estrogen signaling pathway, etc. Animal studies show that JQZF treatment significantly boosts serum C3 and lowers anti-dsDNA antibodies ($P < 0.01$). On the molecular level, JQZF suppresses TLR7 and NF-κBp65 mRNA in spleen B cells, with TLR7 mRNA positively linked to anti-dsDNA titers and negatively to serum C3. Further cellular work demonstrates that JQZF reverses the increased IRAK1 and TRAF6 expression seen after miR146a inhibition. Additionally, post-ERα inhibition, JQZF continues to upregulate miR146a and more significantly reduces TLR7 mRNA expression ($P < 0.01$), pointing to ERα's pivotal role in the miR146a-TLR7 axis. These results indicate JQZF alleviates SLE by adjusting the ERα-miR146a-TLR7 loop, showcasing its mechanism and therapeutic potential for SLE.

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1. Introduction

Systemic lupus erythematosus (SLE) is an intricate autoimmune disease characterized by the production of pathogenic autoantibodies and immune complex deposition, leading to multi-organ involvement. Notably, SLE manifests a pronounced B-cell hyperactivity and proliferation [1]. The disease primarily affects women of childbearing age, and its onset is associated with changes in endogenous hormone levels [2]. Gender and sex hormones influence the susceptibility to SLE [3]. In particular, estrogen stimulates the activation of auto-reactive B cells [4]. The over-activation and damage of B-cells result in the immune system's inability to distinguish between self and non-self antigens, leading to the production of a range of pathogenic antibodies targeting self-antigens, such as anti-dsDNA antibodies, and causing excessive inflammation [5].

Crucially, estrogen receptor alpha (ERα) has a significant interaction with Toll-like receptors (TLRs) in mediating the inflammatory response [6]. Studies conducted on SLE patients have revealed the upregulation of toll-like receptor 7 (TLR7) in peripheral blood mononuclear cells [7], and high TLR7 expression can result in increased B-cells and enhanced autoantibody reactivity [8]. Additionally, there is an increase in the production of anti-DNA and RNA/RNP antibodies, as well as the formation of immune complexes [8]. Although it is believed that ERα and TLRs interact with each other, the specific mechanism of how and whether ERα interacts with TLR7 is still unclear.

MicroRNA (miRNA) plays a pivotal role in the inflammatory response of human immune cells and serves as a vital regulator of the SLE immune process. It has been observed that baseline miRNA profiles differ between males and females [9], suggesting that differences in miRNA expression might contribute to the higher prevalence of SLE in females. Furthermore, miRNAs have been found to be differentially regulated by estrogen levels [10,11]. A comprehensive analysis investigating the interaction between miRNA regulation of SLE etiology and its epigenetic regulatory mechanisms identified miR-146a as a susceptibility gene for SLE and lupus nephritis (LN) [12]. This indicates the potential for miRNA-mediated pathogenic alterations in females with SLE. Some researchers have inferred that the heightened prevalence of SLE among females may be linked to the impact of endogenous estrogens, heightened production of Toll-like receptor gene products, and modified functionality of microRNAs [13].

Conventional immunosuppressants and hormone therapies for SLE are associated with a broad spectrum of adverse effects. Addressing fatigue and other prevalent symptoms in SLE continues to be a significant challenge [14]. In this context, traditional Chinese medicine (TCM) has garnered attention for its efficacy and lower toxicity in managing SLE. Jiedu-Quyu-Ziyin Fang (JQZF) is a Chinese herbal formula that has been validated as effective in the treatment of SLE in China, derived from modifications of "Sheng Ma Bie Jia Tang". It consists of 10 herbs and serves as an empirical formula for the clinical treatment of SLE [15].

In clinical trials, JQZF has been successfully applied in the treatment of SLE in a clinical setting [16]. It is already proven to have notable treatment on SLE by inhibiting the progression and has won the second prize in the China National Science and Technology Progress Award [17], and shows particular promise for SLE patients. Pharmacological studies reveal that JQZF's herbal constituents offer benefits in SLE management. For instance, paeoniflorin, a key JQZF component, modulates immune responses and blocks inflammatory pathways in LN [18]. Dihydroartemisinin and prednisone work in synergy to mitigate SLE symptoms and re-establish Treg/Th17 balance [19]. In animal experiments, JQZF alleviates the symptoms of MRL/lpr mice [20]. A series of previous experimental animal studies confirmed that JQZF effectively inhibits disease activity in MRL/lpr mice by blocking the activation of the IRAK1-NF-κB signaling pathway [20]. Further, it showcases anti-inflammatory and anti-fibrotic properties through the inhibition of NF-κB and α-SMA expression, contributing to LN prevention and treatment [21]. JQZF also impedes TLR9/MyD88 signaling, which reduces the risk of lupus combined with atherosclerosis [22]. Although clinical, pharmacological, and animal studies confirm JQZF's therapeutic potential against SLE, the disease of SLE is complex and many potential mechanisms have not been explored in depth, so further mechanistic validation studies are very necessary.

In summary, JQZF has established its utility both traditionally and in clinical pharmacology for SLE, but the immunological effects of JQZF on key factors such as ERα, miRNA, and TLR7 and their interaction relationships have not been fully understood. This study is designed to elucidate these mechanisms via network pharmacology approaches and empirical validation.

2. Materials and methods

2.1. Bioinformatics approach to predict the targets and miRNAs of JQZF for SLE

Databases: BATMAN-TCM database (http://bionet.ncpsb.org.cn/batman-tcm/), GeneCards database (https://www.genecards.org/), STRING database (https://cn.string-db. org/), mirDIP database (http://ophid.utoronto.ca/mirDIP/), mirtarbase database (https://mirtarbase.cuhk.edu.cn/), miRWalk database (http://mirwalk.umm.uni-heidelberg.de/), miRDB database (http://www.mirdb.org/). Software: R 4.11, R CytoHubba plugin and Cluego plugin, Cytoscape 3.8.2.

The targets of Chinese herbal medicines in JQZF were individually retrieved from the BATMAN-TCM database. The targets were then aggregated to obtain the targets of JQZF. The GeneCards database was searched using "Systemic lupus erythematosus" as the search term to obtain the targets of SLE. Furthermore, overlapping targets of JQZF and SLE were subjected to miRNA prediction using the mirtarbase and mirDIP databases. The miRNAs obtained from the two databases were intersected using R software and visualized using the cytoHubba plugin.

Key miRNAs were entered into miRWalk, miRtarBase, and miRDB databases to get predicted target genes, and the genes obtained from the three databases were intersected using R software, then PPI network analysis was performed in String database, and key target genes were visualized using Cytoscape software. The KEGG pathway analysis of the target genes was performed using the cluego plugin of Cytoscape software to obtain the key signaling pathway.

2.2. Experimental drugs

JQZF is an herbal compound, including Rehmannia glutinosa (Gaertn.) DC (Di Huang) 15 g, Actaea cimicifuga L (Sheng Ma) 9 g, Artemisia annua L (Qing Hao) 12 g, Trionyx sinensis Wiegmann (Bie Jia) 12 g, Oldenlandia diffusa (Willd.) Roxb (Bai Hua She She Cao) 15 g, *Centella asiatica* (L.) Urb (Ji Xue Cao) 15 g, Paeonia anomala L (Chi Shao) 12 g, Coix lacryma-jobi L (Yi Yi Ren) 15 g, Citrus medica L (Fo Shou) 9 g and Glycyrrhiza uralensis Fisch (Gan Cao) 6 g. All JQZF were purchased from Zhejiang University of Traditional Chinese Medicine Herbal Beverage Factory. Our group's preliminary High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometry (LC-MS) experiments verified that JQZF contains a variety of phytochemicals such as glycyrrhizic acid and paeoniflorin [23], and is a standard Chinese herbal compound that has been widely used in clinical practice. JQZF preparation process: First, dry herbs were soaked in double-distilled water for 2 h. Then, the turtle shell was boiled for 30 min with other herbs, followed by an additional hour of boiling. The resulting solution was filtered, and the process was repeated once more for 1 h. The two solutions were mixed and concentrated using rotary evaporation at a water bath temperature of 60 °C and a speed of 2500 rpm until the concentration reached 2 g/mL. Methylprednisolone tablets (specification: 4 mg*30T, product batch number: W08029). Preparation of Glucocorticoid (GC) solution for the animal experiment: Dissolve one methylprednisolone tablet in 4 mL of double distilled water to obtain a suspension of 1 mg/mL. Dexamethasone sodium phosphate injection (produced by Guangzhou Pharmaceuticals, specification: 1 mL:5 mg, product batch number: H44022091). Preparation of GC solution for cell experiments: Dissolve one vial of Dexamethasone sodium phosphate injection (5 mg/mL, molecular weight: 392.461) in 95 mL PBS to obtain a storage solution of 100 mM.

2.3. Experimental animals and animal experiment process

Fifteen MRL/lpr mice were randomly divided into three groups: Model, JQZF, and JQZF + GC, with five mice in each group. Five MRL/MPJ mice were used as the Blank group. The Blank and Model groups were administered single distilled water via gavage. The JQZF group was given JQZF (0.33 g/mL) by gavage, at a dosage of 0.1 mL per 10 g of mouse weight. The JQZF + GC group was administered JQZF liquid (0.66 g/mL, 0.1 mL/20 g) and methylprednisolone tablets solution (1 mg/mL, 0.1 mL/20 g) by gavage. All groups were orally gavaged once daily for 8 weeks. After the treatment period, peripheral blood was collected from the mice, and the serum was separated by centrifugation. The levels of anti-double-stranded DNA (dsDNA) antibodies and complement C3 were measured. Single-cell suspensions of the spleen were prepared and B cells were sorted using CD43 (Ly-48) MicroBeads. Flow cytometry analyzed the expression of CD19 on the sorted B cells and the splenocytes before sorting, using Phycoerythrin (PE)-labeled anti-mouse CD19 antibody. The data were analyzed with FlowJo V11.0 software. The sorted spleen B cells were subjected to transcriptome sequencing analysis. The animal study was approved by the Animal Ethical and Welfare Committee of Zhejiang Chinese Medical University (IACUC-20220523-03), adhering to the internationally recognized Principles for the Use and Care of Laboratory Animals U. S. Guidelines.

2.4. Cell experiment process

For validation of miR146a target genes, Raji cells with stable expression of miR146a were used due to their stable expression. A miR146a inhibitor was transfected into the stable Raji cells, followed by the application of Western Blot analysis to ascertain the protein expression levels of Phospho-Interleukin-1 Receptor-Associated Kinase 1 (P-IRAK1) and TNF Receptor Associated Factor 6 (TRAF6). After JQZF treatment, the mRNA expression of IRAK1 and TRAF6 was measured.

The transfection of Estrogen Receptor Alpha (ERα) into primary spleen B cells was executed successfully, designating these cells for subsequent examinations. Following stable culture, JQZF and JQZF + GC were administered, and ERα was downregulated using shRNA before further administration of JQZF and JQZF + GC. The expression levels of miR146a and Toll-like Receptor 7 (TLR7) mRNA was detected using Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR). The Western Blot technique was employed to evaluate the expression levels of TLR7 and phosphorylated Nuclear Factor kappa-light-chain-enhancer of activated B cells p65 (p-NF-κBp65) proteins.

2.5. RT-qPCR and western blot method to detect the expression of key factors

Total RNA was extracted with RNAiso Plus solution (TaKaRa, Cat# 9109). Following the manufacturer's protocol of the Bio-Rad kit (Bio-Rad, Cat# 1708891), reverse transcription was conducted. TLR7, NF-κBp65, GAPDH, IRAK1, and TRAF6 primers were designed and synthesized by Sangon Biotech (Shanghai, China). The miR-146a/U6 primers were provided by Guangzhou RiboBio (Guangzhou, China).

Cell lysis was performed using RIPA lysis buffer to obtain protein. The protein concentration was determined by a multifunctional microplate reader. SDS-PAGE gel was prepared and Enhanced chemiluminescence and exposure imaging were performed.

2.6. Statistical analysis

SPSS 25 and GraphPad Prism 8 were used to analyze and graph the experimental data statistically. One-way ANOVA was used for comparison among multiple groups, and $P < 0.05$ indicated a statistically significant difference.

3. Results

3.1. Bioinformatics approach to predict the possible targets of JQZF

A total of 1354 JQZF targets and 4075 SLE targets were identified, revealing the top 30 intersecting targets, including SRC, AKT1, TP53, JUN, HDAC1, HSP90AA1, EGFR, CTNNB1, ESR1, RXRA, TNF, FOS, STAT5A, NR3C1, F2, HIF1A, STAT5B, IL4, IL6, NFKB1, PPARG, HSPA8 MED1, PRKCA, AR, AGT, CXCR4, HSP90AB1, IL10, IL13 (Fig. 1A). By analyzing the interaction relationships between the overlapping targets in the String database with a highest confidence setting of 0.900, a Protein-Protein Interaction (PPI) network diagram was obtained. Further analysis using the Cytoscape database revealed that ESR1 is the key target (Fig. 1B). The miRNA prediction analysis was performed on the overlapping targets, and 12 miRNAs were obtained from the intersection of mirtarbase and mirDIP databases (Fig. 1C), namely: hsa-miR-146a-5p, hsa-miR-206, hsa-miR-18a-5p, hsa-miR-7-5p, hsa-miR-205-5p, hsa miR-221-3p, hsa-miR-222-3p, hsa-miR-17-5p, hsa-miR-130a-3p, hsa-miR-139-5p, hsa-miR-155-5p, and hsa-miR-148b-3p (Fig. 1D).

3.2. Predicted target genes of hsa-miR-146a-5p

To further investigate, target gene prediction analysis was performed on hsa-miR-146a-5p. The intersection of target genes predicted by miRWalk, miRtarBase, and miRDB databases was taken, resulting in 20 key genes (Fig. 2A), namely: CARD10, PPP1R11, PRKCE, CCDC6, TIMELESS, PMAIP1, ELAVL1, MR1, RARB, TRAF6, RAC1, SHCBP1, TFDP2, CCR9, COPS8, ERBB4, ROBO1, IFIT3, IRAK1, CFH. PPI network analysis was performed and IRAK1 and TRAF6 genes were found to be the key genes (Fig. 2B). KEGG pathway analysis indicated that NF-κB signaling pathway was the key signaling pathway (Fig. 2C and D). These findings suggest that

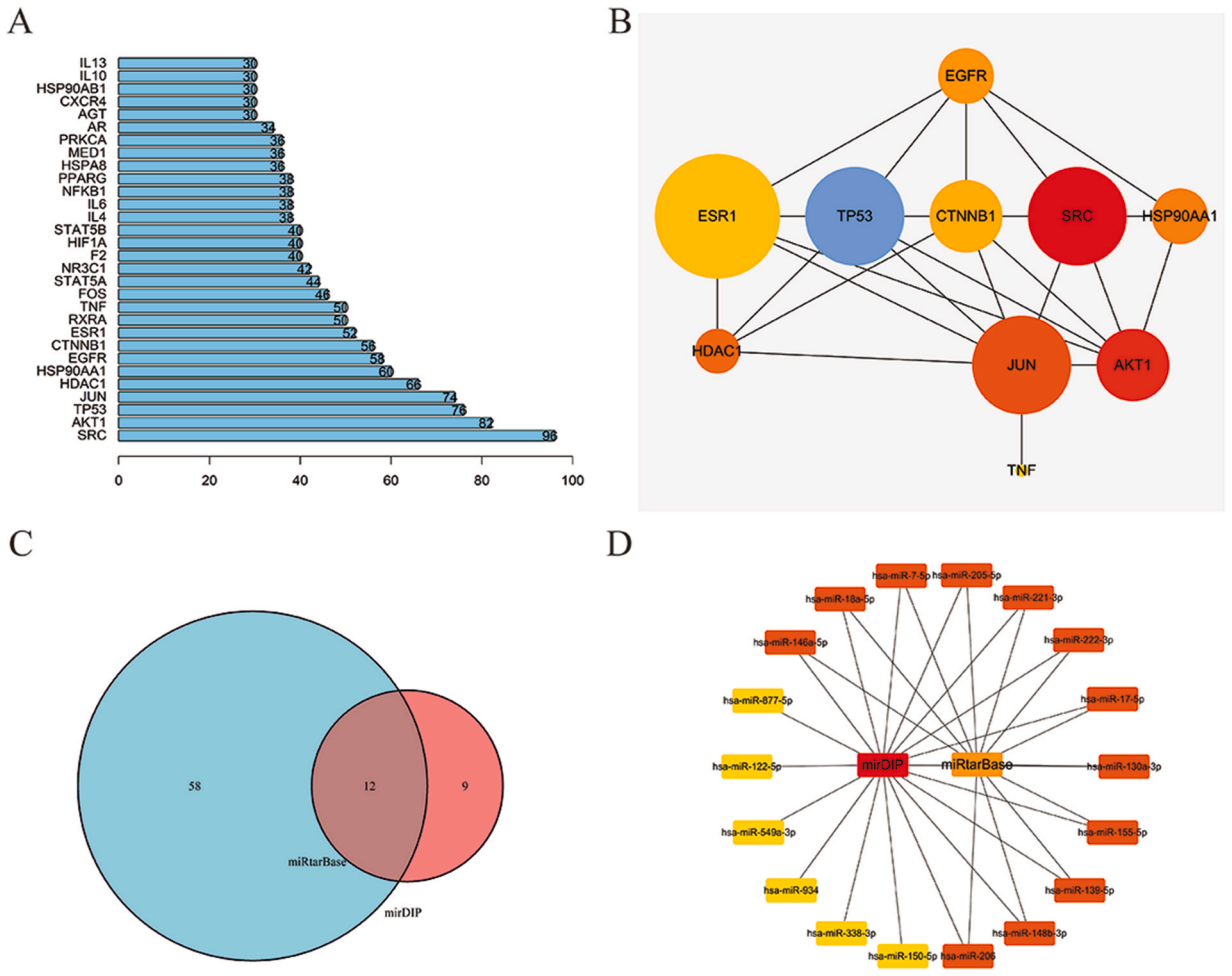

Fig. 1. Predictive analysis of the mechanism of action of JQZF for the treatment of SLE. A The top 30 targets of the intersection of SLE and JQZF. B PPI network diagram of the top 10 intersecting targets. Dots represent the targets of action of JQZF for SLE treatment, and edges represent target-target interactions. C miRNA prediction for the intersecting targets, miRtarBase database, and mirDIP database predicted miRNA intersection map. D Visualization of intersecting miRNAs.

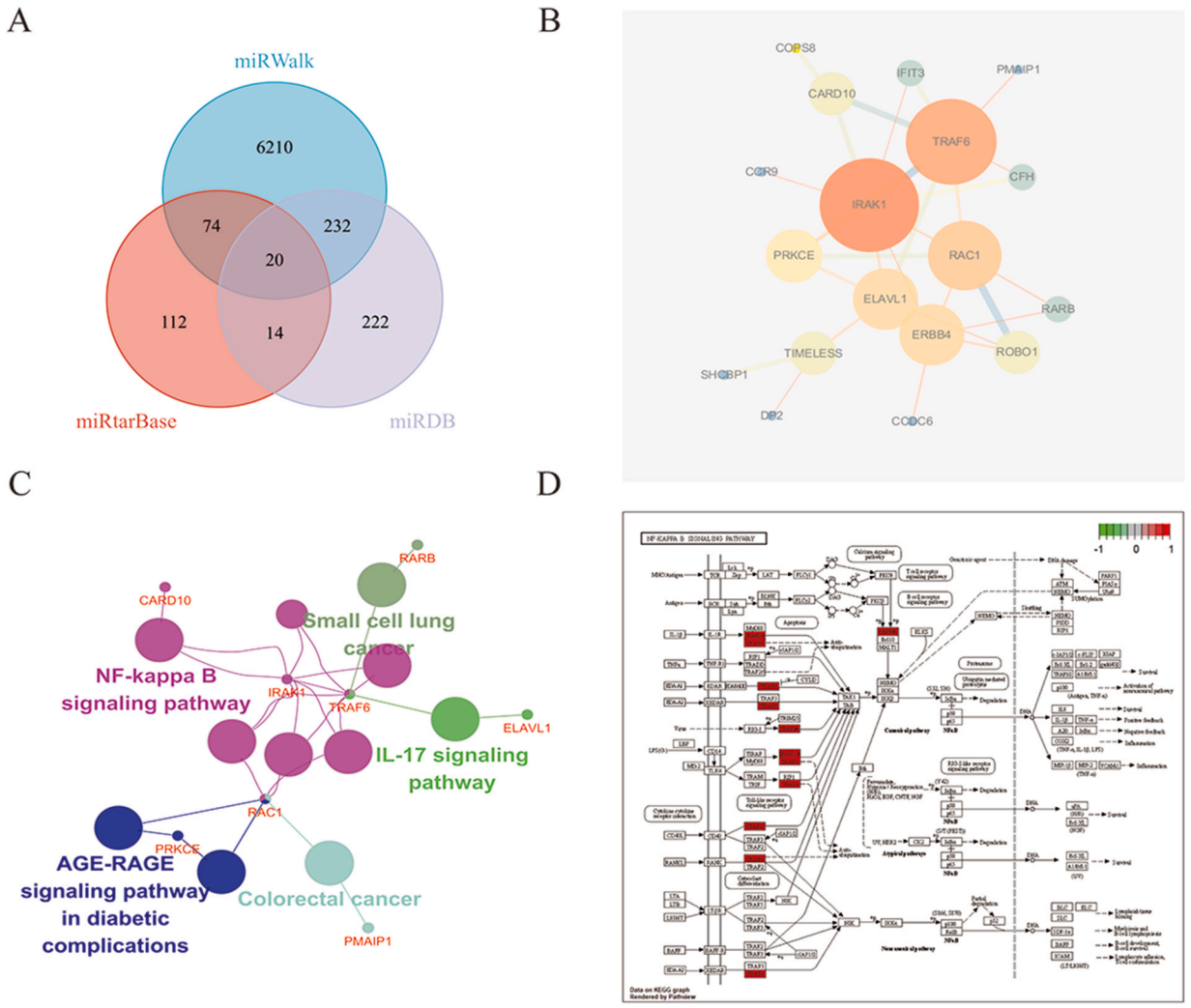

Fig. 2. Prediction of miR146a target genes and related signaling pathways. A Diagram of the intersection of miR146a target genes predicted by miRWalk, miRtarBase, and miRDB databases. B PPI network analysis of common intersection target genes. Dots represent possible target genes of miR146a, and edges represent interactions between genes. C Analysis of KEGG pathway of intersecting targets. D Plot of NF-κB signaling pathway.

hsa-miR-146a-5p may be involved in regulating the NF-κB signaling pathway by targeting IRAK1 and TRAF6, and may have potential implications for related diseases. This prediction provides new insights into the molecular mechanisms of hsa-miR-146a-5p in disease pathogenesis.

3.3. Transcriptome analysis of spleen B cells after administration of MRL/lpr mice

The spleen B cells of each group of experimental animals were subjected to transcriptome sequencing analysis, and it was found that the differences between the JQZF group and the Model group were enriched in Cellular Processes with Phagosome, Tight junction, Regulation of actin cytoskeleton; enriched in Environmental Information Processing with NF -κB signaling pathway, cGMP-PKG signaling pathway, Calcium signaling pathway, Cytokine-cytokine receptor interaction, MAPK signaling pathway; enriched in Human Diseases are Pertussis, Legionellosis, Acute myeloid leukemia, Salmonella infection, Morphine addiction; enriched Metabolism includes Metabolism of xenobiotics by cytochrome P450, Purine metabolism; enrichment in Organismal Systems includes Gastric acid secretion, Salivary secretion, Taste transduction, Complement and coagulation cascades, Insulin secretion (Fig. 3A). Among them, Toll-like receptor signaling pathway, NF-κB signaling pathway, and Estrogen signaling pathway were all included in the top 20 KEGG Enrichments (Fig. 3B). Toll-like receptor signaling pathway and NF-κB signaling pathway showed a downward trend (Fig. 3c).

3.4. The effect of JQZF on SLE disease and core targets

Based on the results of transcriptome sequencing, this study demonstrated that JQZF had a down regulatory effect on Toll-like

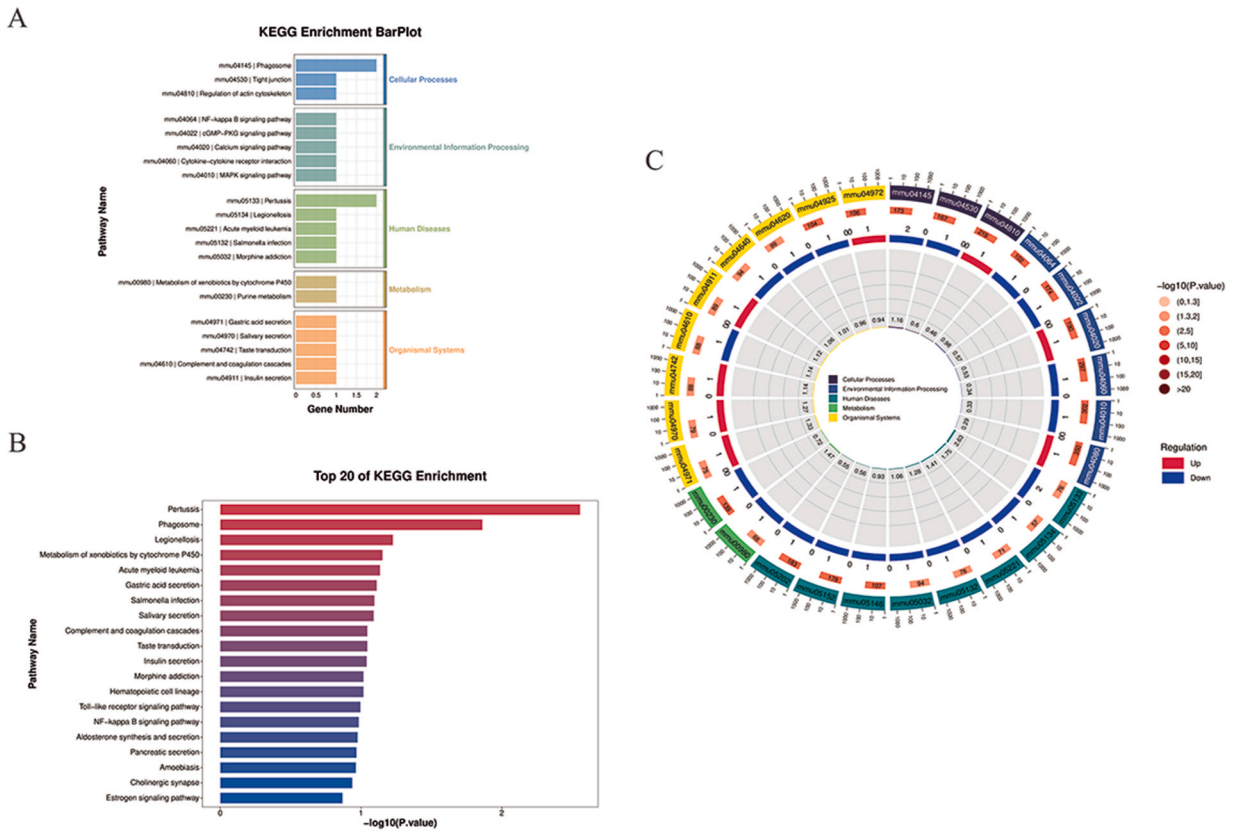

Fig. 3. KEGG enrichment analysis of differential genes in MRL/lpr mice after administration. (A) BarPlot for KEGG analysis of differentially expressed genes in JQZF + GC group and Model group. (B) The first 20 KEGG enrichment pathways of differentially expressed genes in the JQZF + GC group and the Model group. (C) The regulation of key signaling pathways.

receptor signaling pathway and NF-κB signaling pathway. In this study, it was first detected that JQZF could increase the serum C3 level and decrease anti-dsDNA level (Fig. 4A and B). The spleen cells were sorted to obtain B cells, and the purity was tested to meet the experimental needs (Fig. 4C). The validation of the expression of key factors mRNA showed that JQZF could reduce the expression of TLR7 and NF-κBp65 mRNA of B cells (Fig. 4D and E). Further correlation analysis showed that TLR7 mRNA expression was positively correlated with anti-dsDNA antibody and negatively correlated with complement C3 concentration (Fig. 4F and G).

3.5. Effect of JQZF on miR146a and its possible target genes

Based on the results of hsa-miR-146a-5p target gene prediction, the expression of IRAK1 and TRAF6 was examined, and the Western blot results showed that miR146a inhibition group p-IRAK1 and TRAF6 protein expression was upregulated (Fig. 5A), and it can be clear that IRAK1 and TRAF6 are the target genes of miR146a. It was also observed that the IRAK1 mRNA levels were significantly higher in the miR146a inhibition group compared to the Blank group ($P < 0.001$), while the expression of IRAK1 mRNA was decreased in the miR146a inhibition + JQZF group compared with the miR146a inhibition group ($P < 0.01$) (Fig. 5B). The expression of TRAF6 mRNA showed the same trend. It can be seen that JQZF can reverse the change trend of target genes after miR146a inhibition, that is, JQZF plays a role by interfering with miR146a.

3.6. ERα affects the expression of miR146a and TLR7/NF-κB signaling pathway

The previous bioinformatics analysis found that ESR1 is the key target of JQZF in SLE. In the results of B cell transcriptome sequencing, it was also found that the Estrogen signaling pathway is the key signaling pathway. Accordingly, we envision that ERα may play a role in the whole immune loop. After ERα inhibition, JQZF still tends to upregulate miR146a. Following ERα suppression, the trend of downregulation of TLR7 mRNA becomes more pronounced ($P < 0.01$). (Fig. 6A and B). It remains to be noted that the trend of inhibition of TLR7 protein and p-NF-κBp65 by JQZF was also more pronounced after inhibition of ERα (Fig. 6C). This suggests that ERα plays a regulatory role on miR146a-TLR7, and JQZF exerts its effect on alleviating SLE disease through the ERα-miR146a-TLR7 immune loop.

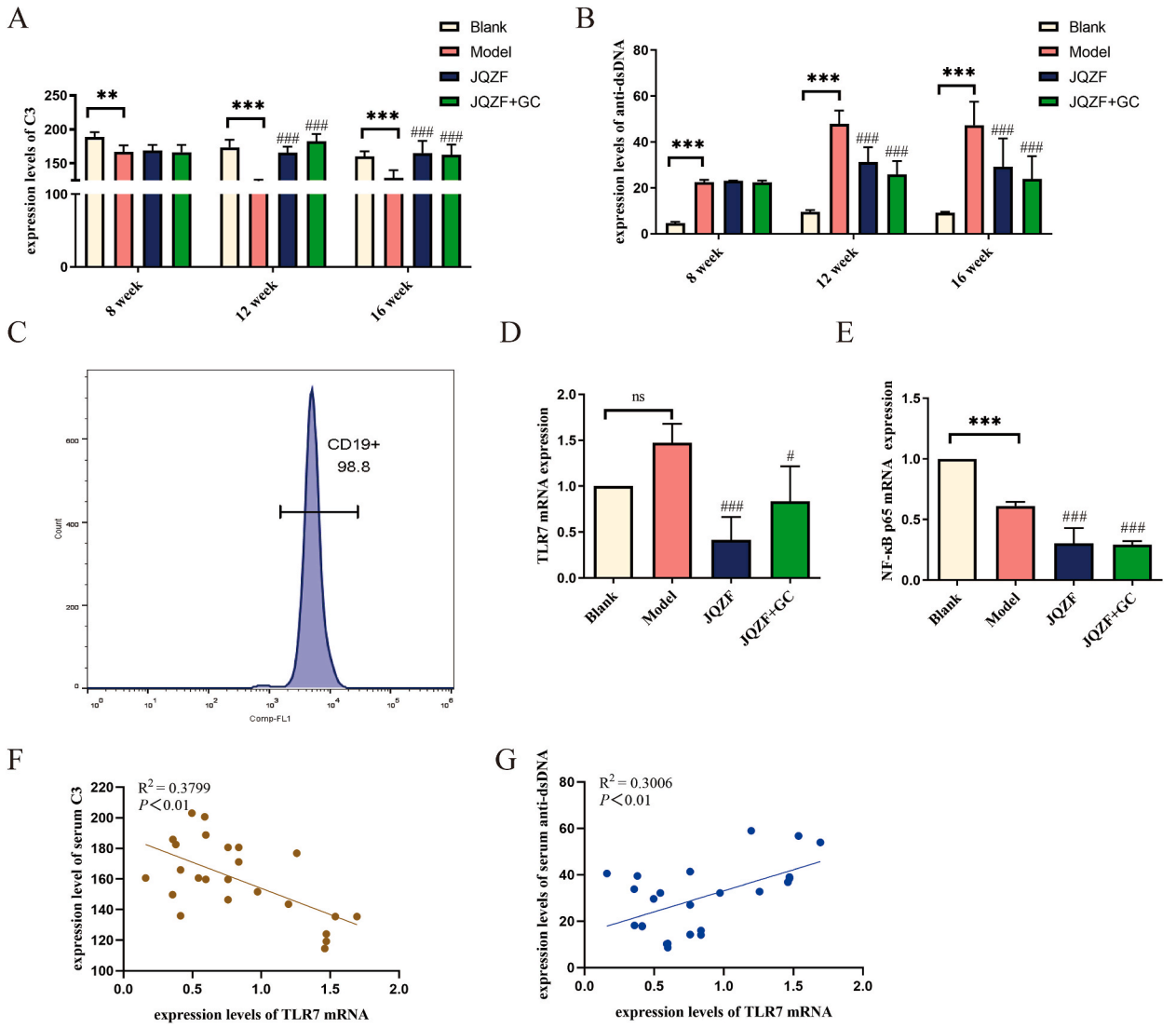

Fig. 4. The effect of JQZF on SLE and expression of the core targets. A Expression of complement C3 in MRL/lpr mice at different dosing times in different dosing groups. B Expression of anti-dsDNA in MRL/lpr mice at different dosing times in different dosing groups. C Sorting efficiency of splenic B cells. D TLR7 mRNA expression levels in splenic B cells in different dosing groups. E Correlation analysis of splenic B cell NF -κBp65 mRNA expression levels. F Correlation analysis of TLR7 mRNA expression levels with serum complement C3 levels. G Correlation analysis of TLR7 mRNA expression levels with serum anti-dsDNA levels.

4. Discussion

SLE is a chronic, multi-system autoimmune disease characterized by autoantibody production [4]. In patients with SLE, the loss of immunological tolerance leads to B cell damage, leading to the inability of the immune system to differentiate between self and non-self antigens, resulting in the production of a series of antibodies against self-antigens and induction of excessive inflammatory responses [24]. The dysfunction of immune cells, particularly the sustained activation of B cells, plays a central role in the pathogenesis of SLE, and B cells can be a therapeutic target for SLE [25,26]. Therefore, finding effective therapeutic methods to regulate B cells and reduce the production of pathogenic autoantibodies is a breakthrough point in conquering SLE. In this study, we selected high-purity B cells after magnetic bead sorting as the experimental object to explore the mechanism of JQZF in treating SLE at the cellular and molecular levels.

TLRs are pattern recognition receptors that play a crucial role in innate immunity [27]. They recognize and bind to different pathogens, activating various signaling pathways that lead to the production of numerous proinflammatory mediators, contributing to the pathogenesis of SLE [28] and affecting SLE susceptibility [29]. TLRs are important molecules that affect B cell activation signaling [30] and can lower the activation threshold of B cells [31]. For example, the expression of TLR7 in B cells of SLE patients during active periods is significantly higher than that of SLE patients during inactive periods or healthy individuals [32]. It can be seen that the TLR7

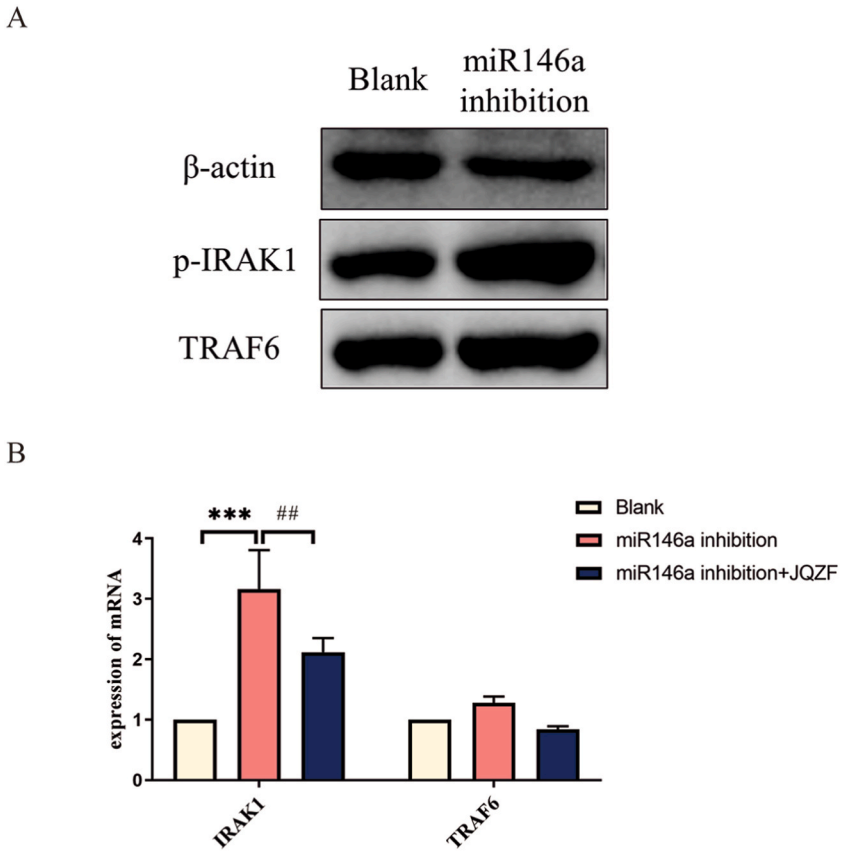

Fig. 5. Expression of key genes after inhibition of miR146a. A Expression of key protein. B Expression of key gene mRNA.

signal in B cells plays an important role in regulating the disease, so inhibiting Toll-like receptors to reduce B cell activation is a key step in treating SLE. In the present study, we showed that JQZF decreased TLR7 mRNA expression, and TLR7 mRNA expression was positively correlated with anti-dsDNA antibody and negatively correlated with complement C3 concentration. It can be seen that JQZF can reduce the expression of TLR7 in B cells, thus exerting a palliative effect.

miRNA has become a major regulator of gene expression [33,34], and it has potential roles in self-immune regulation [35]. A predictive analysis of miRNAs resulted in 12 miRNAs intersecting the mirtarbase and mirDIP databases, including hsa-miR-146a-5p. Immune cells in SLE patients are in an abnormal state, and immune cell dysfunction is associated with dysregulated expression of some miRNAs [36]. In fact, in related studies of SLE, some have suggested that the level of miR146a is negatively correlated with disease activity. Knocking out miR146a in normal mice has led to severe autoimmune symptoms, such as increased levels of autoantibodies and splenomegaly [37]. Meanwhile, some studies have confirmed that IRAK1 is a functional target of miR146a [38]. MiR146a can also have regulatory effects on the activation of NF-κB [39]. Our study revealed that IRAK1 mRNA is a target gene of miR146a and its expression is upregulated when miR146a is inhibited. JQZF was found to upregulate miR146a and downregulate IRAK1 expression in B cells. Previous studies have shown that JQZF can inhibit inflammation in MRL/lpr mice and their bone marrow-derived macrophages by suppressing IRAK1-NF-κB signaling [40]. Our study further supports the important roles of JQZF in regulating immune B cells and miR146a in SLE.

SLE is more prevalent in women of childbearing age and is associated with changes in endogenous hormones [41]. Estrogen plays a crucial role in promoting the activation of autoreactive B cells and the secretion of autoantibodies, contributing to the development of SLE [4]. MiR-146a is decreased in splenic lymphocytes of mice treated with estrogen [42], and it upregulates the expression of NF-κB cytokines [43]. Studies have shown that the targeted loss of ERα in B cells impedes the production of pathogenic autoantibodies and the development of LN in (NZB × NZW) F1 mice [44]. However, it is not clear whether miR146a is regulated by estrogen or whether estrogen can affect Toll-like receptor signaling in SLE B cells. Based on this, to verify the role of ERα in B cells, we used lentivirus transfection to inhibit ERα and then performed JQZF intervention again. We found that after inhibiting ERα, JQZF had a more significant upregulation trend on miR146a and a more significant inhibition trend on TLR7 and NF-κBp65. Therefore, it can be concluded that ERα plays a regulatory role in miR146a-TLR7 pathway.

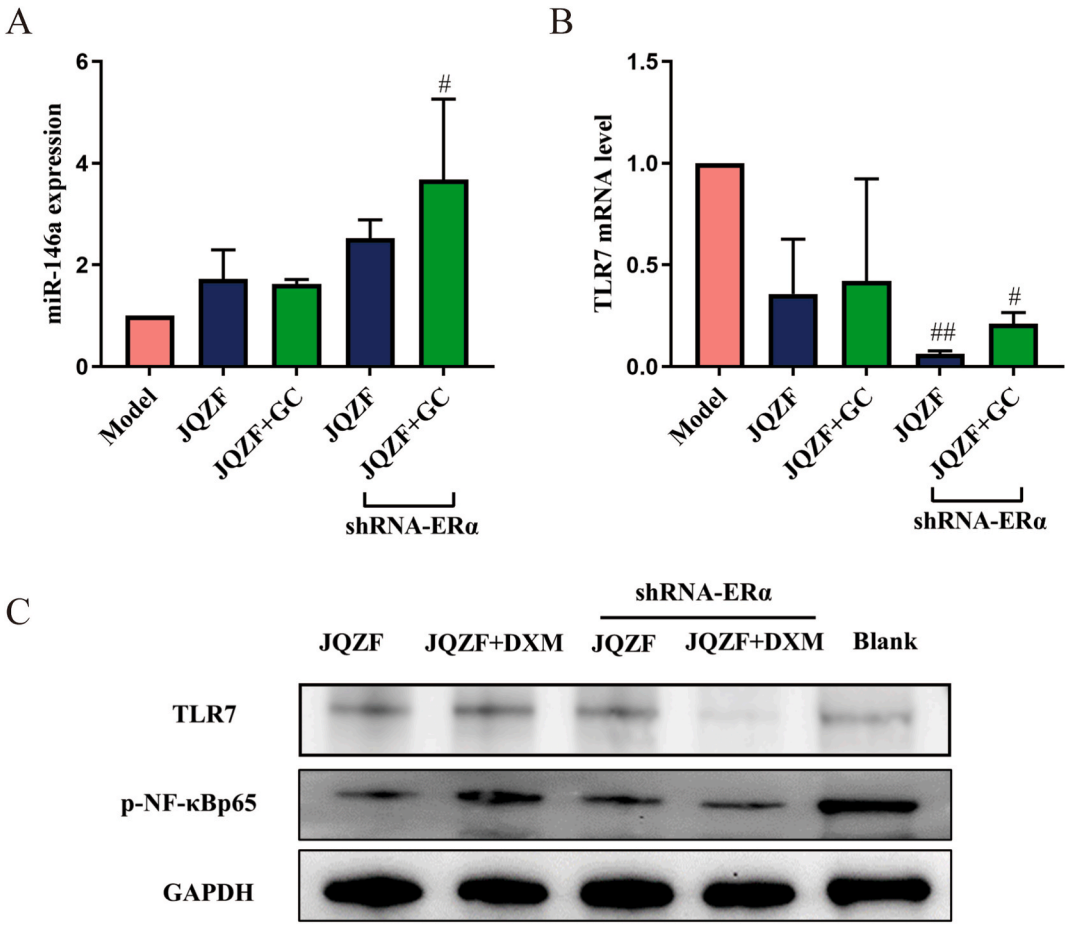

Fig. 6. Effect of JQZF on miR146a and TLR7 signaling pathway before and after ERα inhibition. A Effect of JQZF on miR146a expression before and after ERα inhibition. B Effect of JQZF on TLR7 mRNA expression before and after ERα inhibition. C Effect of JQZF on TLR7/NF-κB signaling pathway protein expression before and after ERα inhibition.

5. Conclusion

In conclusion, this study investigated the effect of JQZF on SLE and its potential mechanism. The experiment demonstrated that JQZF can alleviate SLE by reducing the expression of TLR7 in B cells and downregulating the expression of the miR146a target gene IRAK1. Notably, ERα inhibition also leads to an increase in miR146a, and a more significant suppression of TLR7 and NF-κBp65 by JQZF, further highlighting the critical role of the B cell ERα-miR146a-TLR7 immune pathway in the mechanism of JQZF (Fig. 7). These findings provide a theoretical foundation for the development of novel strategies for treating SLE.

Ethics declarations

The Animal experiment protocol has been reviewed and approved by the Animal Ethical and Welfare Committee of Zhejiang Chinese Medical University (IACUC-20220523-03).

Data availability statement

Data generated and used for analyses of results presented in this manuscript will be provided by the corresponding author on reasonable requests.

CRediT authorship contribution statement

Yi Zhang: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **FengQi Zhang:** Validation, Investigation, Formal analysis, Data curation. **YiYang Zhang:** Validation, Investigation, Formal analysis, Data curation. **MeiJiao Wang:** Validation, Data curation. **Yan Gao:** Validation, Data curation. **HaiChang Li:** Resources, Funding acquisition. **Jing Sun:**

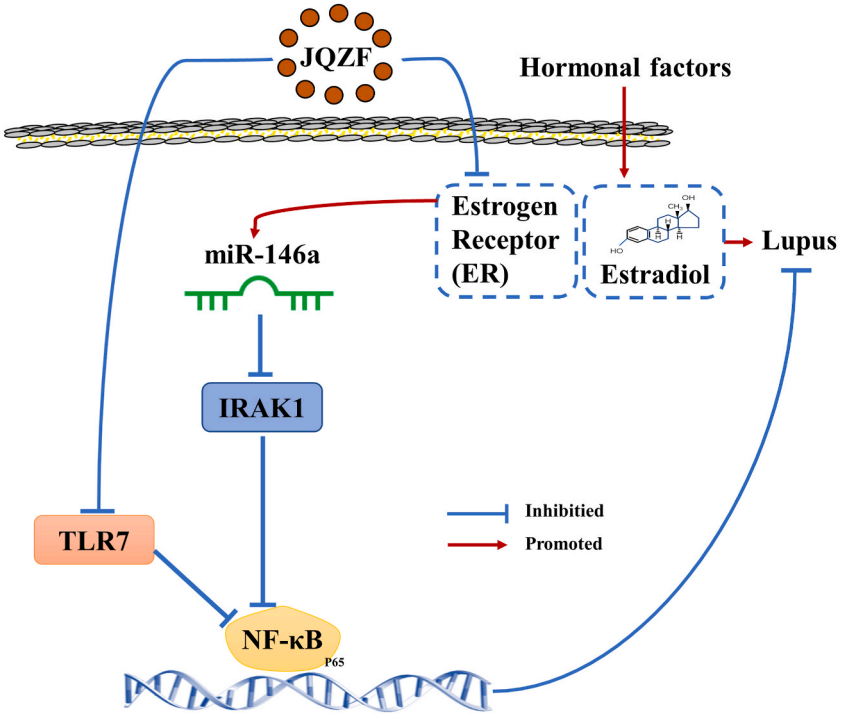

Fig. 7. JQZF plays a role in alleviating SLE through ERα-miR146a-TLR7 immune loop.

Writing – review & editing, Funding acquisition. **ZhiJun Xie:** Writing – review & editing, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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