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Salidroside suppresses cell growth and inflammatory response of fibroblast-like synoviocytes via inhibition of phosphoinositol-3 kinase/threonine kinase signaling in rheumatoid arthritis

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Abstract

Background: Salidroside (Sal) is a natural product commonly isolated from *Rhodiola rosea* L., which has been found to have numerous pharmacological activities (e.g., ameliorating apoptosis and inflammation, and acting as an antioxidant) in various diseases, but its concrete function in rheumatoid arthritis (RA) has not been revealed yet. Here, we aimed to explore the specific role and underlying mechanisms of Sal in RA-fibroblast-like synoviocytes (RA-FLSs).

Methods: Cell counting kit 8 (CCK-8) was used to assess the viability of normal-FLSs and RA-FLSs. Cell apoptosis in RA-FLSs was evaluated by flow cytometry. Western blotting was prepared to examine the levels of apoptosis- and signaling-related proteins. Wound-healing and Transwell assays were conducted to examine RA-FLSs migration and invasion. Enzyme-linked immunosorbent assay (ELISA) was used to assess the effect of Sal on tumor necrosis factor-alpha (TNF- α)-induced inflammation in RA-FLSs. RA animal model was established through complete Freund's adjuvant (CFA) induction, and the histopathological changes in synovial tissues of the rat model were analyzed by H&E staining.

Results: RA-FLSs were treated with 200 μ M Sal for 24 h, and cell viability was significantly suppressed. Sal promoted RA-FLSs apoptosis. The migratory and invasive abilities of RA-FLSs were markedly inhibited by Sal. Sal incubation reduced the levels of inflammatory cytokines interleukin-8 (IL-8), IL-1 β , and IL-6 in RA-FLSs under the stimulation of TNF- α . Subsequently, Sal downregulated phosphorylated phosphatidylinositol-3 kinase (p-PI3K) and protein kinase (p-AKT) expression in RA-FLSs. After the treatment with pathway activator 740Y-P (20 μ M) in RA-FLSs, the promotive effect of Sal on cell apoptosis was reversed, and inhibitory effects of it on cell viability, migration, invasion, and inflammatory response were abolished. Sal inhibited RA development in the CFA-induced rat model.

Conclusion: Sal suppressed cell growth and inflammation in RA-FLSs by inactivating PI3K/AKT-signaling pathways.

Keywords

Synovial tissue hyperplasia · Herbal medicine · Complete Freund's adjuvant · TNF- α · Fibroblast-like synoviocyte migration

Data Availability Statement

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.



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Introduction

Rheumatoid arthritis (RA) is a type of autoimmune disease, and RA patients often suffered from the swelling, stiffness, and undesirable musculoskeletal pain, which can impair the life quality of the RA patients [1]. Hyperplasia of synovial tissues is one of the main RA pathological features, and a large number of activated fibroblast-like synoviocytes (FLSs) exist in them [2–4]. In addition, tumor-like properties including increased proliferation, decreased apoptosis and prolonged survival can be found in RA-FLSs. More importantly, inflammation can be caused in RA-FLSs by producing pro-inflammatory cytokines [5], playing an essential role in RA development. Thus, it is of great significance to find new targets that can affect cell growth and inflammatory response of RA-FLSs, thus, improving the treatment strategy for RA.

Salidroside (Sal) extracted from *Rhodiola rosea* L. has been found to possess various pharmacological activities like anti-inflammatory [6], anti-depressive [7], cardioprotective [8], neuroprotective [9], and anti-apoptotic [10] effects. Many studies have discussed the role of Sal in bone-related diseases, particularly in osteoarthritis. For example, Sal alleviates cartilage degeneration via the nuclear factor-kappaB (NF- κ B) pathway [11]. Sal protects ATDC5 chondrocyte cells against lipopolysaccharides-induced inflammatory response via regulating miR-145 expression [12]. Moreover, its powerful anti-apoptotic, anti-phlogistic and pro-proliferating effects also have been revealed in osteoarthritis [13]. Furthermore, the protective effect of Sal on cognitive dysfunction induced by arthritis in the blood and brain tissues was shown in a previous study [14]. In addition, Liu et al. revealed the inhibitory effect of Sal on gouty arthritis [15]. However, its concrete role and underlying mechanism in RA have not been discussed yet.

It has been widely reported that the inhibition of phosphoinositol-3 kinase/threonine kinase (PI3K/AKT) signaling by molecule drugs is a novel research strategy for RA treatment [16, 17]. PI3K/AKT signaling participates in the growth and apoptosis of RA-FLSs, as well as the secretion of inflammatory factors in the

synovium [18]. The regulatory effect of Sal on PI3K/AKT signaling has been widely reported [19, 20]. Thus, it is necessary to understand the relationship between Sal and PI3K/AKT signaling in RA.

Here, we hypothesized that Sal may exert its pro-proliferating, anti-inflammatory, and anti-apoptotic functions in RA via the PI3K/AKT signaling pathway. A cell model of RA-FLSs and an RA rat model induced by complete Freund's adjuvant (CFA) were used to verify this hypothesis.

Materials and methods

Cell culture and treatment

Human fibroblast-like synoviocytes (normal-FLSs) and human fibroblast-like synoviocytes: rheumatoid arthritis (RA-FLSs) were purchased from Cell Applications (San Diego, CA, USA). Cells were maintained at 37°C in Dulbecco's modified Eagle's medium (DMEM) containing carbon dioxide (5%), foetal bovine serum (FBS) (10%), penicillin (100 U/mL), and streptomycin (100 μ g/mL) (all from Gibco, Grand Island, NY, USA).

Salidroside (purity: 99.79%; MedChemExpress, Shanghai, China) was dissolved in 0.1% dimethyl sulfoxide (DMSO; Sigma, St. Louis, MO, USA) and diluted to the final concentrations. Both normal-FLSs and RA-FLSs were treated with Sal at different concentrations (25, 50, 100, and 200 μ M) for the indicated number of hours (24, 48, and 72 h) to confirm the optimal doses and treatment time using cell counting kit-8 (CCK-8) assay. Meanwhile, the cells in control group were treated with DMSO (0.1%) as vehicle. Finally, RA-FLSs treated with 200 μ M Sal for 24 h were used for the in vitro assays. To detect the effect of Sal on inflammation, RA-FLSs were stimulated with 10 ng/mL tumor necrosis factor-alpha (TNF- α ; Sigma) for 12 h. To conduct the rescue assay, RA-FLSs were treated with pathway activator 740Y-P (20 μ M; MedChemExpress) for 24 h following Sal treatment.

CCK-8 assay

Normal-FLSs and RA-FLSs were seeded in 96-well plates (1×10^4 cells/well) for 24 h. After the indicated treatment of Sal or Sal

with 740Y-P, cells were added with CCK-8 solution (10 μ L; Solarbio, Beijing, China). Then, normal-FLSs and RA-FLSs were further incubated for 24, 48, and 72 h, respectively, to determine viability. Absorbance at a wavelength of 450 nm was measured using a microplate reader (REAGEN, Shenzhen, China).

Flow cytometry

After the 200 μ M Sal treatment for 24 h, RA-FLSs were harvested with trypsin and resuspended in binding buffer (500 μ L) in a centrifuge tube. Subsequently, annexin V-fluorescein isothiocyanate (FITC) (5 μ L) and propidium iodide (PI) (10 μ L) (APPLYGEN, Beijing, China) were added and cultured with the RA-FLSs in the dark for 15 min. Finally, the apoptosis in RA-FLSs was analyzed by flow cytometer (BD Biosciences, San Jose, CA, USA).

Western blot analysis

Proteins were extracted from the treated RA-FLSs by lysis buffer (Beyotime Biotechnology, Shanghai, China) and quantified by bicinchoninic acid (BCA) kit (Beyotime Biotechnology). Subsequently, proteins (25 μ g) were separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred into polyvinylidene difluoride (PVDF) membranes [Millipore, Billerica, MA, USA]). Then, the membranes were blocked with 5% non-fat milk (Sigma) at room temperature for 1 h in Tris-buffered saline (Sigma). Primary antibodies of Bcl-2 (ab182858), Bax (ab32503), cleaved caspase-3 (ab32042), cleaved caspase-9 (FNab01296), p-PI3K (ab278545), PI3K (ab180967), p-AKT (ab38449), AKT (ab131168), GAPDH (ab8245), and horse radish peroxidase (HRP)-conjugated secondary antibody were obtained from Abcam (Shanghai, China) and FineTest (Wuhan, China). Membranes were treated with the primary antibody at 4°C overnight, and then incubated with HRP-conjugated secondary antibody for 1 h at room temperature after washing with phosphate buffer saline (PBS; Sigma). Enhanced chemiluminescence kit (ECL, Millipore) was used to visualize the protein blots. The protein

band intensity was determined using the ImageJ software.

Wound-healing assay

RA-FLSs were seeded in 12-well plate (5×10^5 cells/well) and cultured with 200 μ M Sal for 24 h. Subsequently, a pipette tip was used to make the wounds, and the width of the wounds were observed under a microscope. Then, photographs of each group of cells were taken immediately at 0 h and at 24 h, and the migratory ability of RA-FLSs was analyzed.

Transwell assay

To examine cell migration, RA-FLSs (5×10^4 cells per well) were resuspended in serum-free medium and cultured into the uncoated upper 24-well Transwell chamber with 8 μ m pore size (Corning Inc., Corning, NY, USA). Transwell chamber precoated with Matrigel (40 μ L; BD Biosciences) was used for the invasion assay. Then the DMEM containing FBS (10%) as a chemoattractant was added to the lower chamber. After 24 h of incubation at 37°C, RA-FLSs that had migrated into the lower chambers were fixed in methanol (Sigma) for 15 min and stained with crystal violet (0.1%; Sigma) at room temperature for 30 min. The degree of cell migration and invasion was determined by counting under a microscope (Olympus, Tokyo, Japan).

Enzyme-linked immunosorbent assay (ELISA)

The levels of inflammatory cytokines (IL-8, IL-1 β , and IL-6) in the supernatants of treated RA-FLSs under the stimulation of TNF- α were evaluated using corresponding ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Complete Freund's adjuvant (CFA) animal model

Sprague–Dawley (SD) rats (male; $n = 24$; 220–250 g, Shanghai Experimental Animal Center, Shanghai, China) were housed in their cages ($n = 5$ in each cage) under standard laboratory conditions with free access

to food and water. After 1 week, rats were randomly divided into three groups ($n = 8$ in each group): normal group, CFA group, and CFA + Sal group. Rats in CFA and CFA + Sal groups were immunized with 200 μ g stable emulsion (2 g/ml bovine type II collagen solution emulsified with an equal volume of complete Freund's adjuvant; Sigma). In addition, rats were given 100 μ g booster injection on day 21 to establish the RA models. Rats in the control group were given normal saline only. In addition, rats in CFA + Sal group were treated with Sal (20 mg/kg and day) orally via intragastric tube for 14 days after the establishment of the RA model. At the end, all the rats were euthanized using 10% chloral hydrate (Sigma). The Institutional Animal Care and Use Committee of Qinghai University Affiliated Hospital approved all the animal procedures.

Hematoxylin and eosin staining

The knee joints were removed from the rats on the last day of the experiments. The removed knee joints were fixed in paraformaldehyde (4%), embedded in paraffin, and cut into slices (5 μ m), which were then stained with hematoxylin and eosin (H&E; YEASEN, Shanghai, China) to determine pathological changes. Histopathological assessment was conducted based on a previous study [21], and the score (0–5) for inflammation, synovial hyperplasia, and pannus was then determined.

Statistical analysis

SPSS 17.0 software (IBM, Armonk, NY, USA) was used to analyze all data, which are presented as mean \pm standard deviation (SD). To compare the difference between two groups, Student's *t* test was utilized. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare the significant differences between multiple groups. The nonparametric Kruskal–Wallis rank-sum test was used to analyze the nonnormally distributed data, such as the histopathological score (0–5). The number of samples (n) and biological replicates (N) are shown in figure legends. The differences were considered statistically significant at $P < 0.05$.

Results

Sal inhibits cell proliferation and promotes cell apoptosis in RA-FLSs

Normal-FLSs and RA-FLSs were all stimulated with different doses of Sal (25, 50, 100, and 200 μ M) for 24, 48, or 72 h to evaluate cell viability using CCK-8 assay. We found that 200 μ M Sal treatment reduced cell viability of RA-FLSs most significantly at all of the analyzed time points (24, 48, and 72 h). More importantly, the viability of normal-FLSs had no significant change after the treatment of Sal at any concentrations (25, 50, 100, and 200 μ M) for 24 h, indicating no cytotoxic effect. We thus confirmed that 200 μ M Sal with 24 h of treatment time can be selected for the subsequent experiments (■ Fig. 1a). Flow cytometry revealed that both early and late apoptosis rates in the Sal group were markedly enhanced compared to the control in RA-FLSs (■ Fig. 1b,c). Consistently, the protein level of Bcl-2 (anti-apoptotic protein) was downregulated and the protein levels of Bax, cleaved caspase-3, and cleaved caspase-9 (pro-apoptotic proteins) were upregulated in the RA-FLSs treated with Sal, suggesting that Sal promoted RA-FLSs apoptosis (■ Fig. 1d).

Sal inhibits cell migration, invasion, and inflammation in RA-FLSs

We then further detected the effects of Sal on RA-FLSs migration and invasion. As shown by the wound-healing assay, the migratory ability of RA-FLSs was suppressed by Sal treatment compared to the control group (■ Fig. 2a). Consistently, the Transwell assay showed decreased migrated cells in the Sal group compared to the control (■ Fig. 2b). Subsequently, the effects of Sal on TNF- α -induced inflammation were assessed using ELISA in RA-FLSs. As expected, TNF- α stimulation led to increased levels of IL-8, IL-1 β , and IL-6 in the supernatant of the cells. In contrast, Sal reduced the levels of these inflammatory cytokines significantly, suggesting the anti-inflammatory action of Sal during RA (■ Fig. 2d).

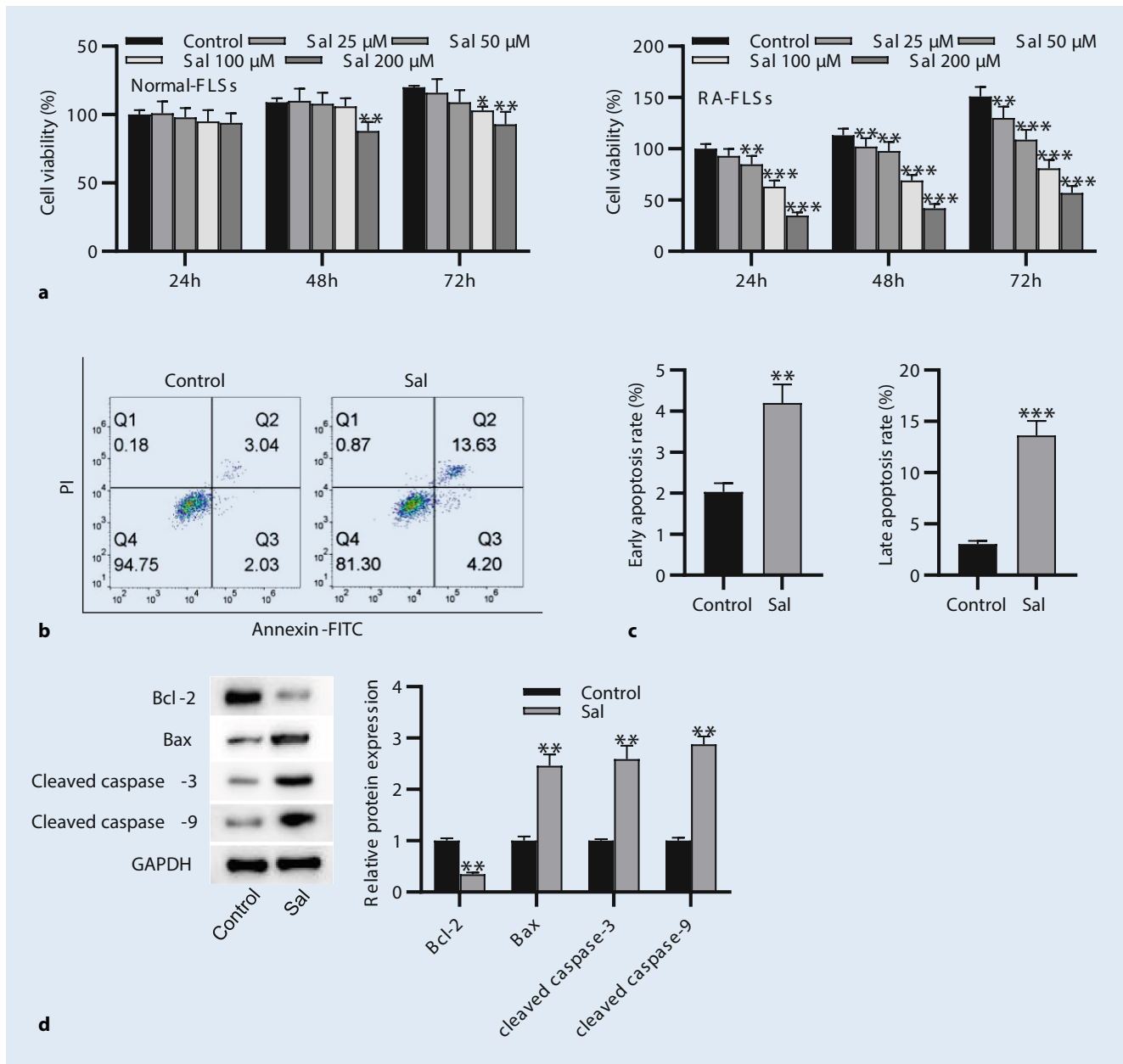


Fig. 1 ▲ Salidroside (Sal) inhibits cell proliferation and promotes cell apoptosis in rheumatoid arthritis fibroblast-like synoviocytes (RA-FLSs). **a** Cell viability was assessed in normal-FLSs and RA-FLSs after the Sal treatments at different concentrations (25, 50, 100, and 200 μM) using cell counting kit-8 (CCK-8) assay. **b,c** Flow cytometry revealed early apoptosis rate and late apoptosis rate in RA-FLSs treated with Sal (200 μM). **c,d** Western blotting revealed the levels of apoptosis-related proteins in RA-FLSs treated with Sal (200 μM). All data are presented as mean ± standard deviation (SD) ($N=3$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Sal inactivates PI3K/AKT signaling pathway in RA-FLSs

Evidence has confirmed the participation of the PI3K/AKT signaling pathway in regulating cell apoptosis and inflammatory process, which has been confirmed as a new target of RA treatment [22]. Thus, we used western blotting to assess the

effect of Sal on this signaling pathway. Surprisingly, Sal significantly downregulated the levels of p-PI3K and p-AKT in RA-FLSs. When the cells were further treated with pathway activator 740Y-P, the levels of p-PI3K and p-AKT were almost restored to the control levels (Fig. 3a). CCK-8 revealed that Sal suppressed the RA-FLSs viability, and 740Y-P promoted it com-

pared with the Sal group (Fig. 3b). Flow cytometry indicated that the increased early and late apoptosis rates induced by Sal in RA-FLSs was obviously inhibited by 740Y-P (Fig. 3c,d). Consistent with that, 740Y-P also enhanced the level of Bcl-2 and restrained the levels of Bax, cleaved caspase-3, and cleaved caspase-9 compared with the Sal group, suggesting

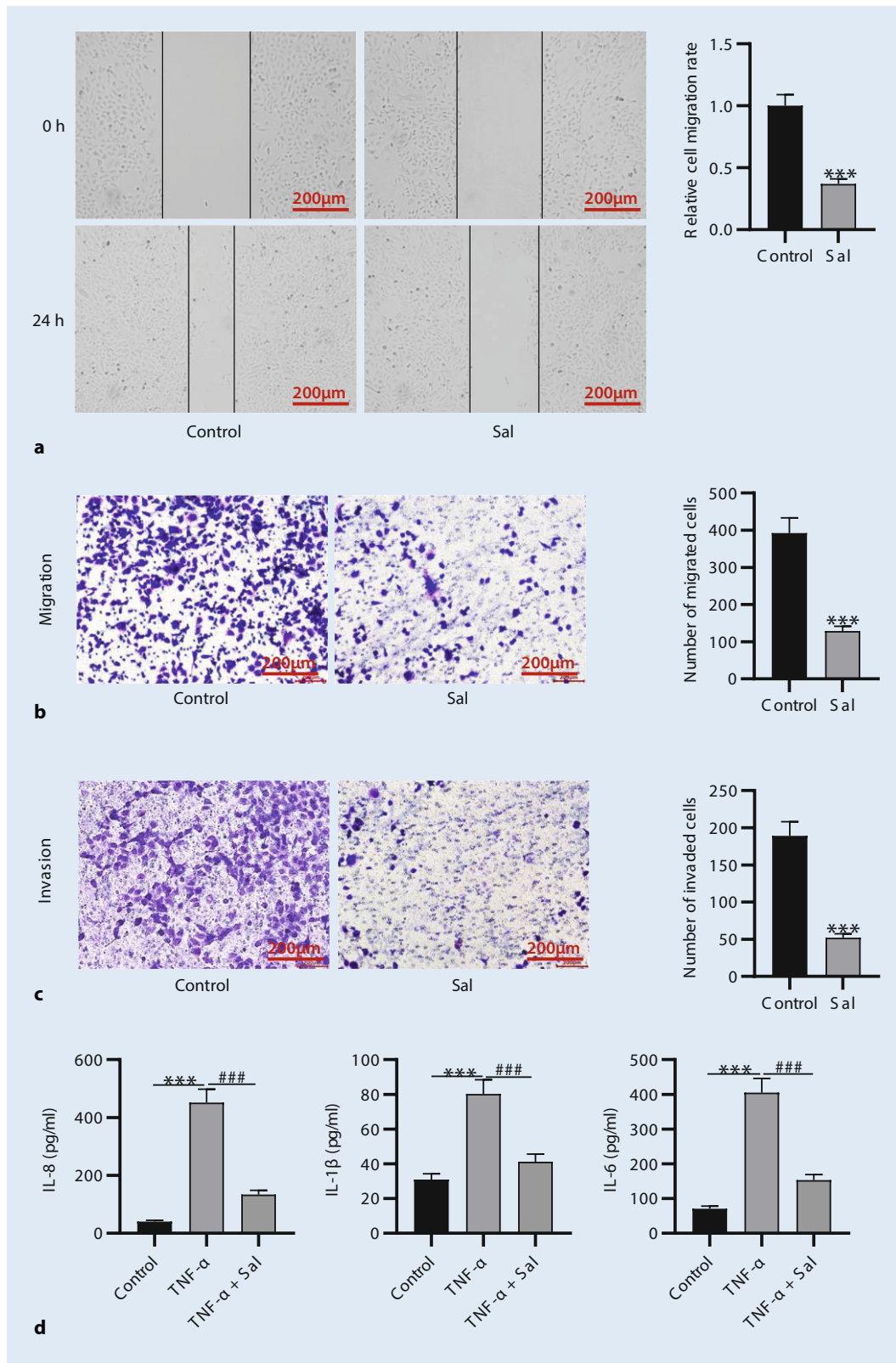


Fig. 2 ◀ Salidroside (Sal) inhibits cell migration, invasion, and inflammation in rheumatoid arthritis fibroblast-like synoviocytes (RA-FLSs). **a** The wound-healing assay was used to analyze the changes in cell migration. **b,c** Transwell assay was used for assessing cell migration and invasion in RA-FLSs treated with Sal (200 μM). **d** Enzyme-linked immunosorbent assay (ELISA) showed the levels of inflammatory cytokines in RA-FLSs stimulated with TNF-α (10 ng/mL) and treated with Sal (200 μM). All data were represented as mean ± standard deviation (SD) (N = 3). *** $p < 0.001$ vs control group; ### $p < 0.001$ vs TNF-α group

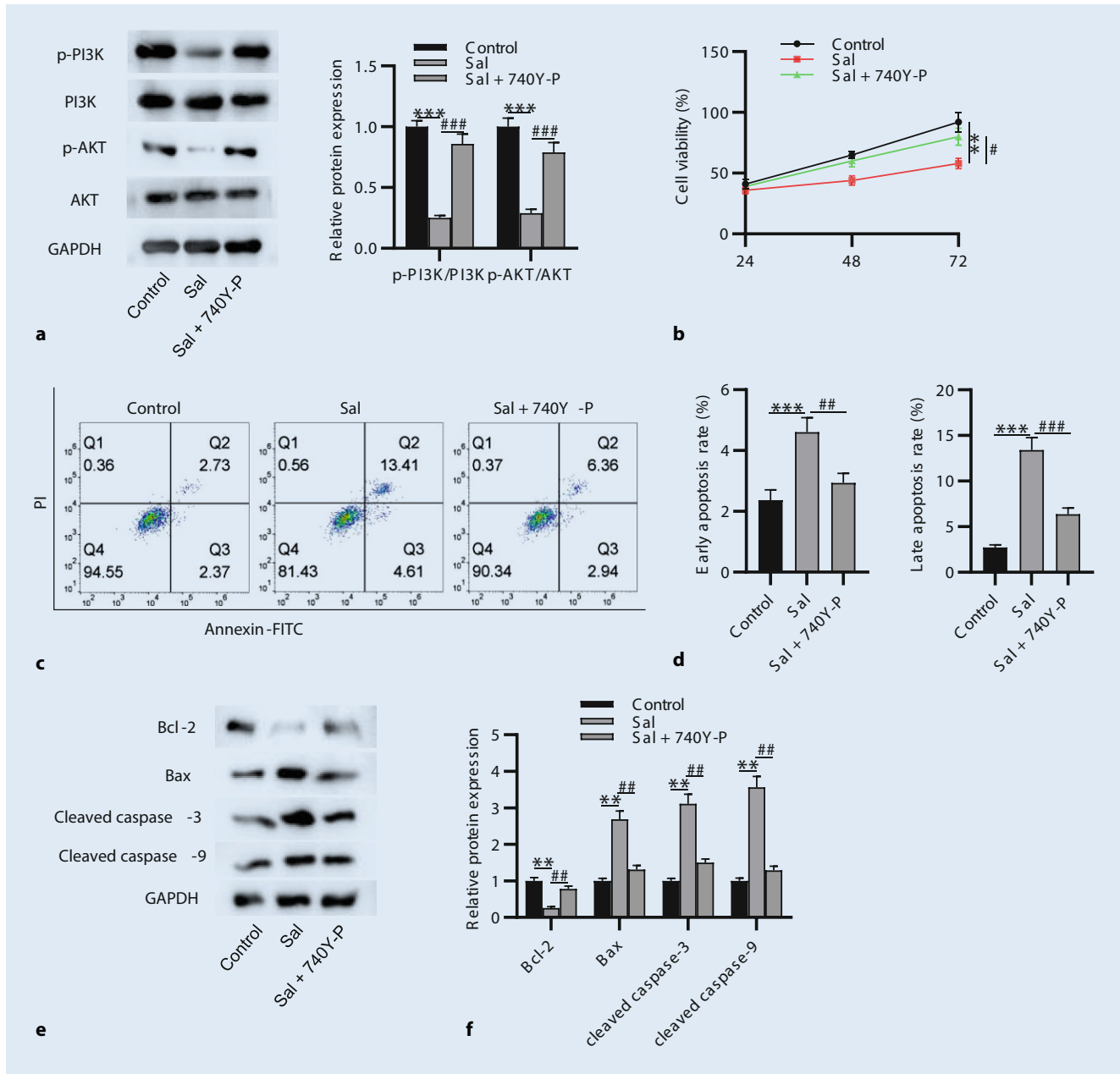


Fig. 3 ▲ Salidroside (Sal) inactivates phosphoinositol-3 kinase/threonine kinase (PI3K/AKT) signaling pathway in rheumatoid arthritis fibroblast-like synoviocytes (RA-FLS). **a** Western blotting was used to examine the levels of signaling-related proteins in RA-FLSs treated with Sal (200 μ M) and signaling activator 740Y-P (20 μ M). **b** Cell counting kit-8 (CCK-8) showed the changes in cell viability. **c, d** Flow cytometry revealed cell apoptosis rate. **e, f** Western blotting presented the expression of apoptosis-related proteins in RA-FLSs treated with Sal (200 μ M) and 740Y-P (20 μ M). All data were represented as mean \pm standard deviation (SD) ($N=3$). ** $p < 0.01$, *** $p < 0.001$ vs control group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs Sal group

that Sal inhibited cell proliferation and enhanced cell apoptosis by inactivating the PI3K/AKT signaling pathway in RA-FLSs (■ Fig. 3e,f).

Sal inhibits cell migration, invasion, and inflammation in RA-FLSs via the PI3K/AKT signaling pathway

To confirm whether the PI3K/AKT signaling pathway influenced the inhibitory effect of Sal on RA-FLSs migration and invasion, RA-FLSs were further treated with Sal and pathway activator 740Y-P was further in-

vestigated to conduct the subsequent experiments. As expected, the reduction in cell migration and invasion after Sal treatment was significantly increased by 740Y-P in RA-FLSs, as shown by Transwell assays (■ Fig. 4a,b). In TNF- α -stimulated RA-FLS, Sal suppressed the levels of inflammatory cytokines IL-8, IL-1 β , and IL-6. However, the inhibition on these inflammatory cy-

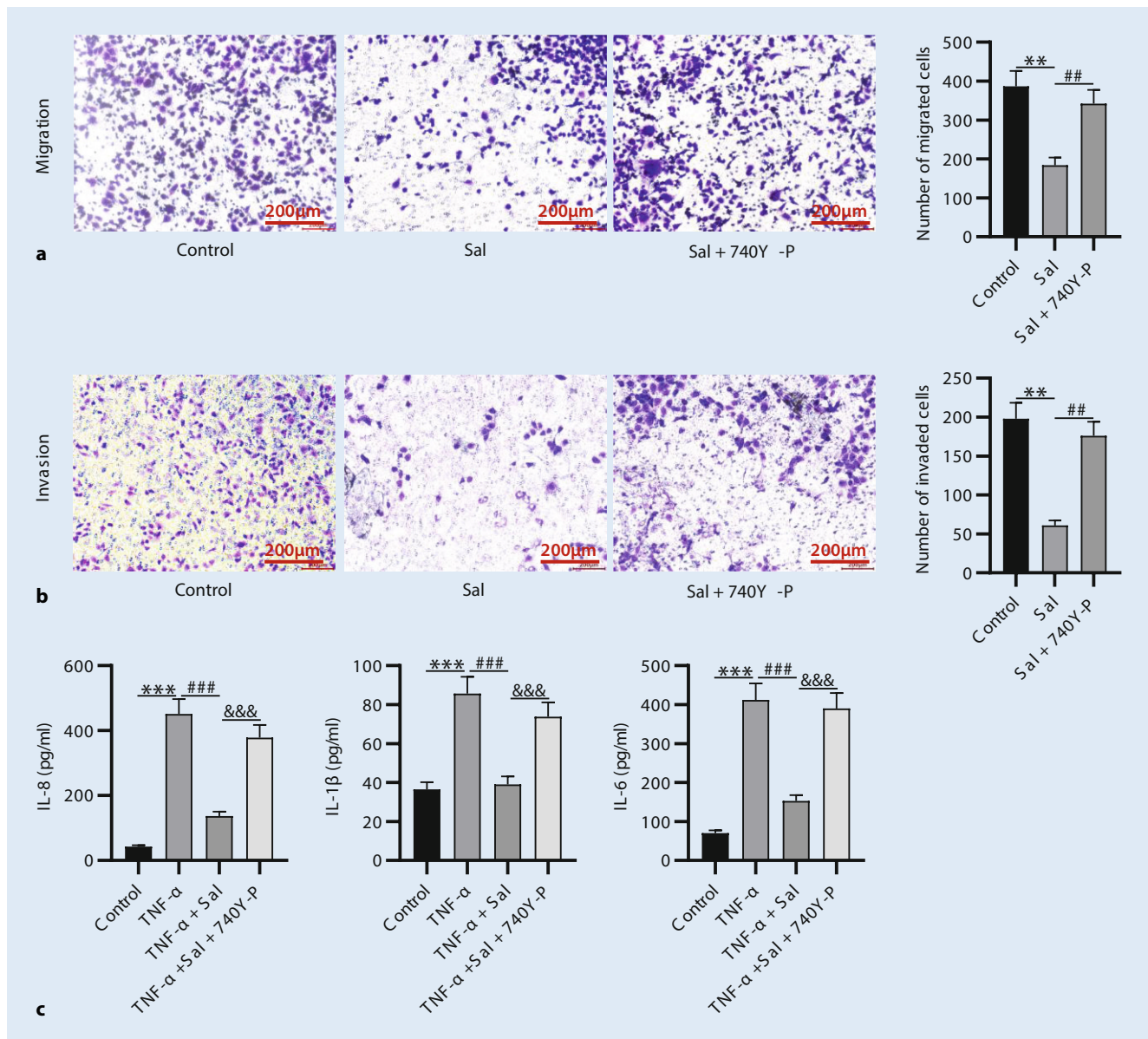


Fig. 4 ▲ Salidroside (Sal) inhibits cell migration, invasion, and inflammation in rheumatoid arthritis fibroblast-like synovio-cytes (RA-FLSs) via the phosphoinositol-3 kinase/threonine kinase (PI3K/AKT) signaling pathway. **a,b** Transwell assay revealed the changes in cell migration and invasion in RA-FLSs treated with Sal (200 μM) and 740Y-P (20 μM). **c** Enzyme-linked immunosorbent assay (ELISA) showed the levels of inflammatory markers in RA-FLSs stimulated with TNF-α (10 ng/mL) and treated with Sal (200 μM) and 740Y-P (20 μM). All data were represented as mean ± standard deviation (SD) (N = 3). ***p* < 0.01, ****p* < 0.001 vs control group; ##*p* < 0.01, ###*p* < 0.001 vs Sal or TNF-α group; &&&*p* < 0.001 vs TNF-α + Sal group

tokines was then abolished by 740Y-P, indicating that Sal had an anti-inflammatory role during RA via the inactivation of the PI3K/AKT signaling pathway (■ Fig. 4c).

Sal alleviates RA development in CFA rat model

To further investigate the role of Sal in vivo, rats were randomly divided into three groups (*n* = 8 in each group): nor-

mal group, CFA group, and CFA + Sal group. According to the results of H&E staining, smooth and clear tissues without inflammatory cell infiltration were presented in normal control group. Inversely, we observed obvious hyperplasia of synovial tissues, increased inflammatory cell infiltration, damaged articular cartilage, and narrowed articular cavity in the CFA group, while the Sal-treated rat model presented significantly improved

histopathological changes (■ Fig. 5a). In addition, the histopathological scores for inflammation, synovial hyperplasia, and pannus in the rat model was evaluated. Apparently, the scores in CFA + Sal group were obviously lower than those in the CFA group, suggesting that Sal had a mitigating effect on RA development in rat model (■ Fig. 5b).

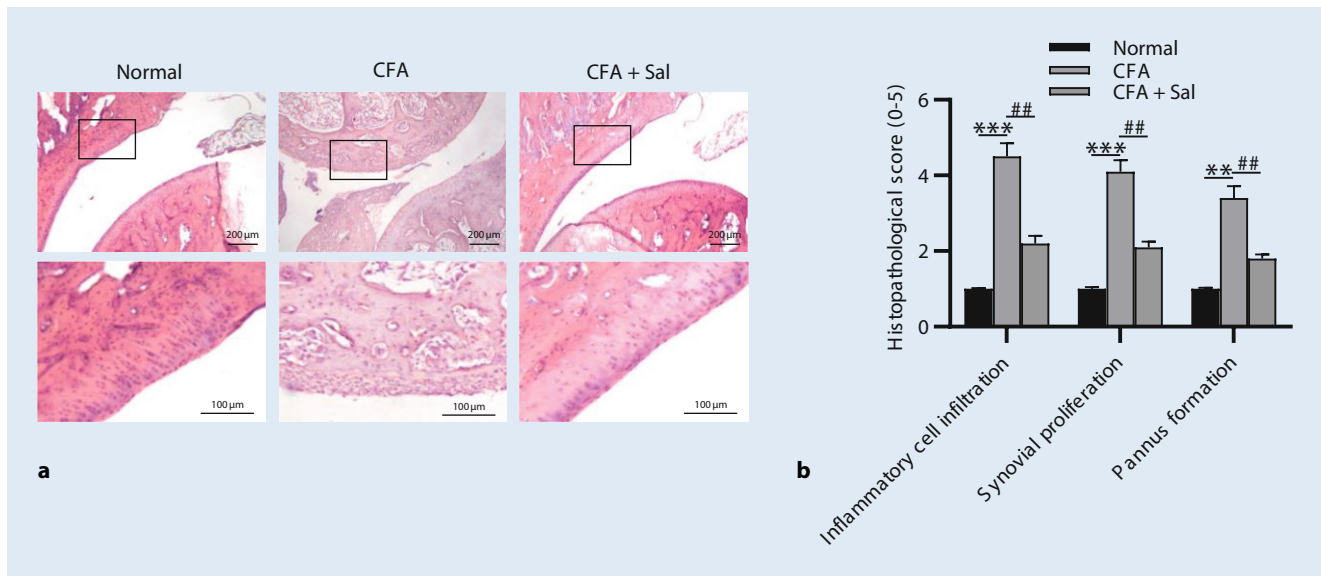


Fig. 5 ▲ Salidroside (Sal) alleviates rheumatoid arthritis (RA) development in Complete Freund's adjuvant (CFA) rat model. **a** Hematoxylin and eosin (H&E) staining of synovial tissues, and **b** histopathological score in normal, CFA, and CFA + Sal groups. All data were represented as mean \pm standard deviation (SD; $n = 8$ in each group). ** $p < 0.01$, *** $p < 0.001$ vs normal group; ## $p < 0.01$ vs CFA group

Discussion

In recent years, many bioactive molecules from traditional Chinese medicine have been revealed to exert potent anti-RA effects through various mechanisms. For example, piperlongumine alleviates RA by inhibiting the proliferation, migration and invasion of RA-FLSs [23]. *Cinnamomi ramulus* is also one of the anti-RA traditional Chinese medicine formulas that has anti-proliferative and migratory effects on RA-FLSs [24]. Eugenol existing in a variety of plants suppresses RA progression by inhibiting TNF- α -induced cell growth and inflammation of RA-FLSs, and promoting RA-FLSs apoptosis [25]. In this study, we for the first time confirmed the anti-RA potential of Sal through the inactivation of the PI3K/AKT signaling pathway.

Normal-FLSs are responsible for keeping the stability and lubrication of the articular cavity by secreting collagen, hyaluronic acid, and fibronectin, thus, playing a crucial role in RA development. In contrast, RA-FLSs present an active proliferative state and impaired apoptosis [26]. Here, Sal significantly suppressed the RA-FLSs viability and promoted the cell apoptosis. More importantly, the protein level of Bcl-2 (anti-apoptotic protein) was downregulated and the protein levels

of Bax, cleaved caspase-3, and cleaved caspase-9 (pro-apoptotic proteins) were upregulated in the RA-FLSs treated with Sal, suggesting the anti-RA potential of Sal.

Emerging evidence has suggested that inhibiting RA-FLSs migration and invasion is a critical therapeutic target for treating RA [27]. Sal has been reported to inhibit the cell proliferation, migration, and invasion activity of various cell types, like RF/6A cells and BV2 microglia [28, 29]. Here, we found that Sal markedly inhibited the migratory and invasive capacities of RA-FLSs. RA is well-known as an inflammatory immune disease [30]. The activated RA-FLS can cause synovial proliferation, inflammation, and joint damage by producing pro-inflammatory cytokines [31]. The anti-inflammatory action of Sal also has been widely demonstrated [32, 33]. Consistently, we found that Sal prominently suppressed the levels of inflammatory cytokines IL-8, IL-1 β , and IL-6 in RA-FLSs under the stimulation of TNF- α , indicating that Sal exerted anti-inflammatory effects during RA.

PI3K/AKT is an important signaling pathway that can regulate various cellular processes, and recent studies highlighted its close relationship with RA development [34, 35]. Sal has been reported to induce

the inactivation of PI3K/AKT signaling, which play a beneficial role in many diseases, including cerebral ischemia-reperfusion injury [19], osteoarthritis [13], myocardial injury [36, 37], and atherosclerosis [38]. In our study, Sal treatment repressed the phosphorylation of PI3K and AKT in RA-FLSs. Rescue assays further revealed that the signaling activator 740Y-P significantly abolished the inhibitory effect of Sal on cell proliferation, migration, invasion, and TNF- α -induced inflammation in RA-FLSs and reversed its promotive effect on RA-FLSs apoptosis, indicating that Sal alleviated RA development by inactivating the PI3K/AKT pathway.

To further prove the anti-RA potential of Sal, the RA rat model was established through CFA induction to conduct in vivo assays. Previous studies have reported many joint pathological changes including swelling, synovial inflammation, and joint damage in the CFA-induced animal model [39]. Similarly, we observed, obvious hyperplasia of synovial tissues, increased inflammatory cell infiltration, damaged articular cartilage, and narrowed articular cavity in CFA group compared with the normal rats. Particularly, Sal significantly inhibited synovial hyperplasia, reduced inflammatory cell infiltration, and alleviated

cartilage damage relative to the CFA group, which is further evidenced by prominently reduced histopathological score for inflammation, synovial hyperplasia, and pannus in Sal-treated rats.

However, some limitations still exist in this study. For example, the underlying mechanism of Sal has not been investigated *in vivo*. In the future, a more representative *in vivo* tissue samples will be prepared, and more complete *in vivo* experiments will be designed to further detect the role of Sal in RA.

Conclusion

Sal had an inhibitory effect on cell growth and inflammation in rheumatoid arthritis fibroblast-like synoviocytes (RA-FLSs) by inactivating the phosphoinositol-3 kinase/threonine kinase (PI3K/AKT) signaling pathways and mitigated inflammatory symptoms and destruction of synovial in the Complete Freund's adjuvant (CFA)-induced RA rat model. The results of our study provide a new idea for investigating RA development, and Sal may become a novel target to treat RA.

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Author Contribution. Y. Qin was the main designer of this study. Y. Qin and J. Su performed the experiments and analyzed the data. Y. Qin and J. Su drafted the manuscript. Y. Qin and J. Su read and approved the final manuscript.

Declarations

Conflict of interest. Y. Qin and J. Su declare that they have no competing interests.

All procedures performed in studies involving human participants or on human tissue were in accordance with the ethical standards of the institutional and/or national research committee and with the 1975 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. The Institutional Animal Care and Use

Committee of Qinghai University Affiliated Hospital approved all the animal procedures.

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Unterdrückung von Zellwachstum und entzündlicher Reaktion fibroblastenartiger Synoviozyten durch Salidroside via Inhibition des Phosphatidylinositol-3-Kinase-/Proteinkinase-Signalwegs bei rheumatoider Arthritis

Hintergrund: Salidroside (Sal) ist ein natürliches Produkt, das gewöhnlich aus *Rhodiola rosea* L. isoliert wird und bei dem zahlreiche pharmakologische Wirkungen (z. B. Verbesserung von Apoptose und Inflammation sowie Wirkung als Antioxidans) bei verschiedenen Krankheiten festgestellt wurden, aber seine konkrete Funktion bei rheumatoider Arthritis (RA) ist noch nicht dargelegt worden. In der vorliegenden Arbeit war es das Ziel, die spezifische Rolle und die zugrunde liegenden Mechanismen von Sal bei mit RA assoziierten fibroblastenartigen Synoviozyten (RA-FLS) zu untersuchen.

Methoden: Zur Prüfung der Lebensfähigkeit von normalen FLS und RA-FLS wurde das Zellzählungssystem CCK-8 („cell counting kit 8“) eingesetzt. Zellapoptose bei RA-FLS wurde mittels Durchflusszytometrie gemessen. Mit Westernblots wurde der Gehalt an apoptose- und signalwegassoziierten Proteine bestimmt. Zur Untersuchung der RA-FLS-Migration und -Invasion wurden Wundheilungs- und Transwell-Assays durchgeführt. ELISA-Tests wurden zur Bestimmung der Wirkung von Sal auf durch Tumornekrosefaktor-alpha (TNF- α) induzierte Inflammation in RA-FLS verwendet. Ein RA-Tiermodell wurde mithilfe der Induktion durch CFA („complete Freund’s adjuvant“, komplettes Freund-Adjuvans) etabliert, und die histopathologischen Veränderungen im Synovialgewebe des Rattenmodells wurden anhand der Hämatoxylin-Eosin(HE)-Färbung analysiert.

Ergebnisse: RA-FLS wurden mit 200 μ M Sal für 24 h behandelt, und die Zelllebensfähigkeit wurde so wesentlich unterdrückt. Sal förderte die RA-FLS-Apoptose. Die Migrations- und Invasionsfähigkeit von RA-FLS wurde durch Sal deutlich inhibiert. Durch Sal-Inkubation reduzierten sich die Werte der inflammatorischen Zytokine Interleukin-8 (IL-8), IL-1 β und IL-6 in RA-FLS unter der Stimulation von TNF- α . In der Folge wurde durch Sal die Expression von phosphorylierter Phosphatidylinositol-3-Kinase (p-PI3K) und Proteinkinase (p-AKT) in RA-FLS downreguliert. Nach Behandlung mit dem Signalwegaktivator 740Y-P (20 μ M) in RA-FLS kehrte sich der fördernde Effekt von Sal auf die Zellapoptose um, und seine inhibitorischen Effekte auf die Zelllebensfähigkeit, -migration, -invasion und entzündliche Reaktion wurden aufgehoben. Sal inhibierte also die RA-Entstehung im CFA-induzierten Rattenmodell.

Schlussfolgerung: Sal unterdrückte Zellwachstum und entzündliche Reaktionen in RA-FLS durch Inaktivierung des PI3K/AKT-Signalwegs.

Schlüsselwörter

Hyperplasie des Synovialgewebes · Pflanzliche Medizin · Vollständiges Freund’sches Adjuvans · TNF- α · Fibroblastenähnliche Synoviozytenmigration