The anti-angiogenesis mechanism of Geniposide on rheumatoid arthritis is related to the regulation of PTEN

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Received: 8 January 2022 / Accepted: 10 March 2022 / Published online: 7 April 2022

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Abstract
Rheumatoid arthritis (RA) is a systemic immune disease characterized by joint inflammation and pannus. The nascent pannus contributes to synovial hyperplasia, cartilage, and tissue damage in RA. This study aims to explore the therapeutic effect and potential mechanism of Geniposide (GE) on RA angiogenesis, involving the participation of phosphate and tension homology deleted on chromosome ten (PTEN) and downstream pathways. Clinical manifestations, synovial pathomorphology, microvessel density, and the level of angiogenesis-related factors were used to evaluate the therapeutic effect of GE on adjuvant-induced arthritis (AA) rats. The proliferation, migration, and tube formation of human umbilical vein endothelial cells (HUVECs) indicate the degree of angiogenesis in vitro. Lentivirus over-expression of PTEN was employed to elucidate the potential mechanism. The results showed that GE improved the degree of arthritis and angiogenesis in AA rats. The expression of PTEN was decreased significantly in vivo and in vitro, and over-expression of PTEN improved the biological function of HUVECs to inhibit angiogenesis. GE inhibited the proliferation, migration, and tube formation of HUVECs and plays an anti-angiogenesis role in vitro. Mechanism study showed that PTEN expression was increased and p-PI3K and p-Akt expression was decreased with GE treatment. It suggests that GE up-regulated the expression of PTEN and inhibited the activation of PI3K-Akt signal, which plays a role in inhibiting angiogenesis in RA in vivo and in vitro.

Keywords
PTEN · Geniposide · Angiogenesis · Rheumatoid arthritis · Human umbilical vein endothelial cells · Phosphatidylinositol 3-kinase

Introduction
Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic synovial inflammation and pannus. Angiogenesis, as an early key event in the pathogenesis of RA, promotes leukocyte recruitment and synovial hyperplasia and forms aggressive pannus tissue, thereby destroying adjacent cartilage and bone tissues, which is one of the main reasons for RA with long-standing incurability (MacDonald et al. 2018; Wang et al. 2021a). In RA disease states, neovascularization not only recruits leukocytes to induce an immune imbalance that aggravates inflammation, but also provides oxygen, nutrients, and signaling molecules for synovial tissue to maintaining the sustained proliferation of fibroblast-like synoviocytes (FLSs) and increasing the recruitment of inflammatory cells into the tissue, ultimately leading to synovitis and tumor-like proliferation of FLSs. FLSs are triggered by hypoxic environment or pro-inflammatory cytokines produced by neighboring macrophages, producing inflammatory cytokines and/or angiogenic mediators, such as growth factors, hypoxia-inducible factors, chemokines, matrix metalloproteinases, and adhesion molecules (Elshabrawy et al. 2018; Balogh et al. 2019). A tightly regulated network of angiogenic mediators converges to activate the interaction and signal crosstalk between cells, which pathologically change the function of endothelial cells to aggravate the angiogenesis of joint synovial. Malignantly proliferating synovial tissue induces angiogenesis via mediators and signaling molecules, and new vascular structures are
in turn required for the constant expansion of synovial tissue, ultimately forming a pannus that synergistically promotes the progression of RA.

Angiogenesis involves a variety of physiological events such as development, tissue repair, and wound healing, but uncontrolled neovascularization contributes to angiogenic diseases, including RA. Angiogenesis is the process of forming new blood vessels from pre-existing vessels, which is dependent on the interaction of vascular endothelial cells (VECs), fibroblasts, macrophages, and extracellular matrix under the regulation of by pro/anti-angiogenic mediators, thus participating in RA pathogenesis (Elshabrawy et al. 2015). Under physiological conditions, blood vessels remain quiescent and rarely form new branches; Whereas under pathological conditions, VECs continuously and orderly form new blood vessels under the regulation of angiogenic mediators and signaling axes. First, VECs are activated under the stimulation of angiogenic signals, such as inflammatory factors, hypoxia, and immune imbalance, and produce matrix metalloproteinases (MMP) to contribute to the degradation of basement membrane and extracellular matrix, which supports VECs proliferation and migration to form new tubular structures. Subsequently, blood vessels are stably developed to complete blood flow and transport functions under the action of pro-angiogenic factors such as angiotensin I (Ang I) (Naito et al. 2020; Alam et al. 2017). In RA, the predominance of pro-angiogenic factors over endogenous anti-angiogenic factors triggers angiogenesis to induce elevated inflammatory cell infiltration and synovial hyperplasia. On the contrary, inhibition of joint neovascularization can effectively alleviate synovial inflammation and pannus formation, which has been supported by numerous studies in animal models of arthritis. Therefore, targeting angiogenesis caused by functional changes of VECs in RA has become an important target for RA treatment.

In addition to angiogenic factors or extracellular signals, the genetic changes and mutations in genes have been found to trigger the process of angiogenesis. Phosphate and tension homology deleted on chromosome ten (PTEN) was first known as a tumor suppressor gene, which is involved in various cellular processes including cell proliferation, survival, angiogenesis, and tumor growth by regulating intracellular signaling (Xue et al. 2018; Liu et al. 2020; Zhang et al. 2020). PTEN, as a negative regulator of phosphatidylinositol-3-kinase (PI3K), regulates PI3K-Akt signal, and participates in angiogenesis. The direct evidence is that the extensive sprouting of new blood vessels and the enlargement of original blood vessels can be observed by the over-expression of PI3K and Akt by RCAs retroviral vector (Jiang et al. 2000). In addition to inhibiting PI3K activation, some studies have found that PTEN regulates the expression of VEGF by activating JNK and ERK signals and participates in the process of angiogenesis, which provides evidence for the important role of PTEN in regulating cell growth, migration, and angiogenesis (Liu et al. 2020; Li et al. 2019; Lu et al. 2020).

Geniposide (GE) is a kind of iridoid glycoside obtained from Gardenia jasminoides Ellis by modern technology of traditional Chinese medicine, which has pharmacological effects of anti-inflammatory and anti-angiogenesis (Ran et al. 2021). Previous work of our group focused on the therapeutic effect of GE on experimental arthritis and found that GE regulates the VEGF level in FLSs and restores the dynamic balance of pro/anti-angiogenic factors in adjuvant-induced arthritis (AA) rats (Wang et al. 2021b; Sun et al. 2020). The purpose of this study is to explore the role of PTEN in the angiogenesis of AA rats and the relationship between downstream PI3K-Akt signal and the anti-angiogenesis of GE in vivo and vitro.

Materials and methods

Drugs and reagents

Geniposide is a white powder with 98% purity and was purchased from DiBai Biotechnology Co., Ltd (24512-63-8, Shanghai, China). Freund’s complete adjuvant (FCA) was obtained from Sigma (SLCD4457, St. Louis, United States). Tripterygium Glycosides (TG) were obtained from Huitian Biopharmaceutical (11158434, Fujian, China). TNF-α was supplied by Novus Biologics (F10301041, United States). PTEN inhibitor Bpv (HOpic) (S8651) and PI3K inhibitor LY294002 (S1105) were obtained from Selleck Chemicals (Texas, United States). Fetal bovine serum (FBS) and Dulbecco’s modified Eagle’s medium (DMEM) were purchased from Thermo Scientific (Hudson, NH, United States). PTEN over-expression lentiviral vector was obtained from Hanbio Biotechnology (Shanghai, China). kFluor488 Click-it Edu imaging detection kit was obtained from KeyGEN BioTECH (KGA331-100, Nanjing, China). Rabbit anti-PTEN antibody (ab170941) was purchased from Abcam (Cambridge, United Kingdom). Rabbit anti-PI3K antibody (#4257), anti-AKT antibody (#4691), anti-p-PI3K antibody (#17366), and anti-p-AKT antibody (#4060) were purchased from Cell Signaling Technology (Boston, United States). Monoclonal antibodies against CD31 (66065-2-lg) and β-actin (66009-1-lg) were obtained from Sanying Biotechnology (Wuhan, China).

Animals and cells

Healthy Sprague–Dawley (SD) rats treated with FCA at 0.1 mL by subcutaneous administration of the left posterior paw to induce adjuvant arthritis, an animal experimental model of RA. Rats without paw swelling were given 0.05 mL FCA supplement before the 7th day. The model...
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was evaluated after 17 days, including arthritis index, paw swelling, and mobility score, and oral administration was continued for a week. Normal control and model group were given the same dose of normal saline. The specific experimental design is shown in Fig. 1. Male SD rats of specific pathogen free (SPF) grade were used in this experiment and weighed 160–190 g. The quality testing unit is Shandong Animal Experimental Center, and the ethics report is issued by the experimental animal ethics committee of Anhui University of traditional Chinese medicine. Human umbilical vein endothelial cells (HUVECs) were provided by BeNa Culture Collection (BNCC, Beijing, China) (No 347734) and grown in an epithelioid adherent manner.

Evaluation of AA rat model

Arthritis index, paw swelling, and mobility score were used to evaluate the model. The arthritis index was judged according to the degree of joint swelling, and the specific criteria were as follows: 0 point indicating normal without swelling; 1 point indicating slight swelling and erythema of ankle joint; 2 points indicating slight swelling and erythema from ankle joint to toe joint or metacarpal joint; 3 points indicating moderate swelling and erythema from ankle joint to toe joint or metacarpal joint; 4 points indicating severe swelling and erythema from ankle joint to toe joint. The maximum score of each rat was 12. Paw swelling was measured with a Volume Meter (ZL-002, Sanying Biotechnology, China) every 3 days after the appearance of inflammation, and the degree of foot swelling was calculated ($\Delta \text{mL} = \text{foot volume after modeling} - \text{foot volume before modeling}$). Mobility score was performed every 3 days by the non-participants’ personnel. Each rat was placed in a box with a certain movement space and observed for more than 30 s. The specific scoring criteria are as follows: 0 point for only lying down; 1 point for crawling; 2 points for standing but difficulty walking; 3 points for walking but difficulty running; 4 points for normal walking.

Histopathological assessment

The synovial tissue was fixed in 4% paraformaldehyde tissue solution (P0099-500 mL, Beyotime, China), dehydrated, and embedded in paraffin. The paraffin blocks were sectioned at 5 μm by a microtome. The sections were stained with hematoxylin (C0105S, Beyotime, China) for 4 min after deparaffinization with xylene and washed with absolute ethanol. The basic dye hematoxylin makes the nucleus blue and purple. Then, the sections were differentiated in 1% hydrochloric acid ethanol for 20 s to wash out excess hematoxylin. Finally, the sections were stained for 90 s with eosin dye, which is red for the cytoplasm and extracellular matrix. The sections were sealed with neutral gum after dehydrated with ethanol and permeabilized with xylene and observed under the microscope (OLYMPUS BX51, Germany) for pathological characteristics of synovial tissue.

Immunohistochemistry

Synovial tissue was first fixed with formalin and then embedded in paraffin to maintain cell morphology. After PBS washing, tissues were incubated with 3% $\text{H}_2\text{O}_2$ for 15 min to block non-specific background staining caused by endogenous peroxidase. Then, the QuickBlock™ immunostaining blocking solution (P0260, Beyotime, China) was incubated at room temperature for 10 min to block non-specific background staining. Anti-CD31 (1:100) (66065-2-lg, Sanying Biotechnology, China), anti-VEGF (1:150) (120 mg/kg) for 1 week. After treatment, the serum and synovial tissue of each group were taken for in vitro experiment. FLSs were cultured and participated in the experiment in vitro with HUVECs.}

Fig. 1 Experimental flow. AA rats model was established on the left posterior paw by single subcutaneous injection of FCA (0.1 mL) at day 1. The model was evaluated from day 7 and the rats with successful modeling were orally given TG (10 mg/kg) and GE (30, 60,
(PA5-85171, Thermo Scientific, United States), and anti-PTEN (1:100) (ab170941, Abcam, United Kingdom) were incubated overnight at 4 °C. The sections were incubated for 30 min with horseradish peroxidase-conjugated secondary antibody (abs20002, Absin, China). DAB chromogen (P0202, Beyotime, China) incubate for 5 min protected from light for color development. The sections were finally counterstained with hematoxylin for 3 min and rinsed with distilled water. The CD31-positive cells clustered into brown as a single microvessel. The average optical density of positive staining was quantified as the relative expression level of the target protein by Image J software analysis.

**Enzyme-linked immunosorbent assay (ELISA)**

The level of an angiogenic factor in serum of AA rats was measured by VEGF specific ELISA kit according to the manufacturer’s protocol (JL21369, J&L Biological, China). The sample was dropped onto a 96-well plate with a specific VEGF antibody to produce a specific binding reaction. Then, the sample was colored with enzyme-containing secondary antibody and enzyme substrate. The absorbance was determined at 450 nm to quantify the samples with a microplate reader (SpectraMax iD3, Molecular Devices, United States). Six replicates were set for each sample.

**Cell culture**

HUVECs were cultured in high-glucose DMEM containing 10% FBS and 1% penicillin–streptomycin solution (PBS) (100 ×). To induce inflammatory conditions, HUVECs stimulated by TNF-α were selected to establish an inflammatory model in vitro. In addition, according to the previous protocol, the cell co-culture technology in vitro has also been applied to better simulate the inflammation in vivo and compare with TNF-α stimulation alone. The specific method was that FLSs derived from synovial tissue of AA rats by in vitro culture were treated with TNF-α (10 ng/mL) for 24 h. The conditioned medium (FLSs-CM) was collected and diluted to different concentrations with DMEM (0%, 5%, 25%, 50%, 75%, and 95%) to stimulate HUVECs for 24 h. The optimal stimulation concentration was selected for experiments.

**Infection and validation of lentivirus**

PTEN over-expression lentiviral vectors were obtained from Hanbio Biotechnology (Shanghai, China). Lentivirus infection of HUVECs induced over-expression of PTEN, and an empty GFP vector (Lv-GFP) was used as a control. After infection, the results of over-expression were detected by Immunofluorescence and quantitative real-time polymerase chain reaction (RT-qPCR) (Roche Lightcycler 480, United States).

**Immunofluorescence staining**

HUVECs were seeded on the round coverslip at a density of 10^5 cells/mL. After 4% paraformaldehyde fixation, 0.5% TritonX-100 permeabilization, and BSA blocking, staining with rabbit anti-CD31 primary antibody was performed overnight. Goat anti-rabbit IgG (H+L) conjugated Cy3 (550076, ZEN-BIO Science, China) was used as a secondary antibody. The nuclei were stained with 4, 6-diamidino-2-phenylindole (DAPI), and the slides were made after dropping anti-quenching reagent (PD131-25, Beyotime, China). Finally, the slides were photographed and analyzed under a fluorescence microscope (OLYMPUS BX51, Germany).

**Edu proliferation assay**

HUVECs were seeded on the round coverslip in 48-well plates at a density of 10^5 cells/mL. Different concentrations of GE or inhibitors Bpv were administered after incubation in the DMEM medium containing 10% FBS for 24 h. The following protocols were performed by kfluor488 Edu cell proliferation detection kit (KeyGEN BioTECH, Nanjing, China). After successful staining, the coverslip was then coated with nail polish on the slide. Finally, it was imaged and analyzed under a fluorescence microscope (OLYMPUS BX51, Germany).

**Cell cycle analysis**

HUVECs were treated with different concentrations of GE or Bpv to analyze the cell cycle when growing to a density of 50%. The cells were digested and fixed in 70% ethanol overnight at 4 °C. The cells were resuspended in PBS the next day, and stained with 400 μL propidium iodide (PI) solution (50 μg/mL) (Solarbio, P8080, Beijing, China) and 100 μL RNase A (100 μg/mL) (Solarbio, R1030, Beijing, China) for 30 min in the dark. Finally, it was analyzed by flow cytometry (Beckman Coulter, FC500) and FlowJo software (Version 10.6.0).

**Cell counting kit-8 assay**

The cells were planted in 96-well plates at the density of 10^5 cells/well and cultured in 37 °C incubator for 24 h. Six replicate wells in each group were treated with TNF-α (0, 1.25, 2.5, 5, 10, 20 ng/mL) (Deng et al. 2021) or FLSs-CM (5%, 25%, 50%, 75%, 95% CM) (Wang et al. 2021b) and incubated for 24 h. Then, 10 μL of CCK-8 solution (G4103, Servicebio, China) was added to each well and continued to culture for 2 h. CCK8 reagents are reduced by dehydrogenases in cells to a highly water-soluble yellow formazan dye product that is proportional in quantity to the number of viable cells. The optical density (OD) at 450 nm was
measured with a microplate reader (SpectraMax iD3, Molecular Devices, the United States), and the cell proliferation rate was calculated based on OD450 values.

### Wound-healing analysis

The wound-healing experiment was used to determine the migration ability of cells. HUVECs were seeded into 6-well plates at a density of 6 × 10⁵ cells/well, and cells were cultured to adhere and grown to a density of 90%. The cells were scratched vertically with 200 μL sterile pipette and ruler, leaving a vertical line. The slipped cells were washed, and photographed under microscope observation. This time point was set at 0 h. Next, TNF-α (10 ng/mL) or GE (25, 50, 100 μM) were treated and photographed after incubation for 6, 12, and 24 h, respectively. The scratch area at different time points was measured by Image J software, and the rate of wound healing was calculated to represent the migration ability of cells.

### Vertical migration analysis

HUVECs (10⁵ cells/mL) were seeded in the upper chamber of a polycarbonate transwell chamber (Corning, United States) with 8 μm pore membranes to evaluate the vertical migration ability of cells. The upper chamber medium was replaced with the low concentration FBS (5%) medium containing TNF-α (10 ng/mL) or GE (25, 50, 100 μM), and the lower chamber was the medium containing 10% FBS. After 24 h of culture, the chamber was removed and washed with PBS once. The cells that did not pass through the membrane were carefully wiped off with a cotton swab, and the cells under the membrane were fixed with 4% paraformaldehyde. The membrane was dried naturally and then stained with crystal violet (G1014, Servicebio, China) for 20 min. Finally, the membrane was fixed on a glass slide and observed under an inverted microscope (Leica DMI6000B, Germany). The number of migrating cells was analyzed by Image J software.

### Tube formation assay

Before the experiment, the 96-well plate and sterile pipettes were pre-cooled at −20 °C in advance, whereas Matrigel (Corning, United States) was thawed at 4 °C. 50 μL of Matrigel was taken in a 96-well plate and incubated for 30 min to solidify in a 37 °C incubator. Cell suspensions made of different groups were seeded into 96-well plate containing Matrigel at a density of 2 × 10⁴ cells/mL. Images were taken by a microscope after 24 h of incubation (Leica DMi6000B, Germany). The number of tubes and the length of branches were calculated by Image J software to represent the lumen forming ability of cells.

### Western blot analysis

HUVECs of different groups grown to 80% were washed three times with ice-cold PBS. 500 μL RIPA lyase (P0013B, Beyotime, China) containing 1% PMSF protease inhibitor (ST506, Beyotime, China) was lysed and then centrifuged at 12,000 rpm at 4 °C for 30 min. The supernatant was collected and the total protein was quantified by a BCA protein quantification kit (EC0001, SparkJade, China). The equivalent protein samples were separated by 8–10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (EC0003, SparkJade, China) and transferred to PVDF membranes (Biosharp, Shanghai, China) under the constant current condition of 200 mA. The membrane was blocked in 5% (w/v) skimmed milk for 2 h to block non-specific protein binding. The membrane was incubated with primary antibody at 4 °C overnight after washing with Tris-buffered saline containing Tween-20 (TBST) (ED0010, SparkJade, China). After that, it was incubated with goat anti-rabbit or mouse IgG horseradish peroxidase-conjugated secondary antibody (ZB-2306, ZSGB-BIO, China) for 1 h at room temperature. The levels of each protein were normalized to β-actin. The protein bands were exposed with the alpha view SA system (protein simple, California, United States) and analyzed by Image J software.

### RT-qPCR assay

Total RNA from different groups of HUVECs was extracted using Trizol reagent (G3013, Servicebio, China). The concentration of RNA was determined, and samples with purity (A260/A280) between 1.8 and 2.1 were selected for the next step. The extracted total RNA was reverse transcribed into cDNA using the EasyScript® All-in-One First-Strand cDNA synthesis kit (AE341-02, Transgen Biotech, China). The expression of the target gene was relatively quantified by SYBR RT-PCR in Lightcycler 480 system (Roche, United States). PCR conditions were 95 °C for 10 min, 40 cycles of 95 °C for 20 s, and finally 55 °C for 30 s. The relative mRNA levels were obtained by the 2−ΔΔCT method using GAPDH as an internal reference gene. Primer design and synthesis were supported by Sangong Biotech (Shanghai, China). The primers’ sequences are as follows. PTEN forward: GTT GAT GTT TATTTTTTTTAAGTGG and reverse: TATCAAATCTAT TTTAAACCCCAAT; PI3K forward: ATCGACCTCAC TTGGGGA and reverse: CAATATCTTCTGGCCGCGG CT; AKT forward: TGGAGTTGTGTGGACAGTGAAC and reverse: AGGTACAGATGATCCCATGCGG.

### Statistical analysis

The experimental data are expressed by mean ± standard deviation (SD) and statistically analyzed by SPSS 23.0.
software. The significance of differences between groups was compared by one-way ANOVA. In all results, $P < 0.05$ or 0.01 was considered statistically significant.

**Results**

**GE improved the symptoms of joint inflammation in AA rats**

SD rats were injected with FCA for 7 days to induce an AA model. AA rats showed swelling (SW) and joint stiffness on the left hind limb, while arthritic nodules (AN) appeared at the ankle (Fig. 2A). From the 7 days to the 17 days after

![Fig. 2](image)

**Fig. 2** GE improved symptoms and synovial histopathological of AA rats. A The paw observation of AA rats showed swelling (SW) and arthritic nodules (AN). B Effects of GE on arthritis index in AA rats. C Effects of GE on paw swelling in AA rats. D Effects of GE on mobility score in AA rats. E Effects of GE on the pathomorphology of synovium in AA rats. The red arrows indicate synoviocytes (S), angiogenesis (A), and inflammatory cells (I) respectively. Data are represented as mean ± SD ($n=8$). *$P < 0.05$, **$P < 0.01$ versus control group; *$P < 0.05$, **$P < 0.01$ versus AA group.
modeling, the arthritis index and paw swelling gradually increased, while mobility score decreased, indicating that the modeling was successful. The arthritis index and paw swelling were significantly decreased and mobility score was increased in AA rats after treatment with GE and TG, indicating that GE effectively alleviated joint inflammation in AA rats (Fig. 2B–D). Synovial pathomorphology showed that the synovium of normal rats was composed of 1 or 2 layers of synovial cells overlaid on the surface of connective and adipose tissue without angiogenesis and inflammatory cell aggregation. On the contrary, synovial tissues of AA rats showed 3–8 layers of hyperplastic synoviocytes (S), with a large number of inflammatory cells (I) infiltrated than that in the AA group, and the inflammatory cell infiltration and vascular proliferation were relatively reduced (Fig. 2E). It further illustrated that GE alleviated the joint inflammation of AA rats, including the histopathological changes.

**GE inhibited angiogenesis in vivo in AA rats**

The brown stained areas shown by immunohistochemical results were CD31-positive expression, and quantification as microvessel density (MVD) was used to represent the level of angiogenesis in vivo. As shown in Fig. 3A, the number of CD31-positive microvessel in the AA group was increased compared with the control group, but decreased significantly after GE and TG treatment. MVD quantification was consistent with the above results (Fig. 3B). The level of classical angiogenic factor VEGF represents the level of angiogenesis. Immunohistochemical and quantitative results showed that the tissue expression of VEGF was increased in the AA group, while it was decreased significantly after GE treatment (Fig. 3A, C). Similarly, the level of VEGF in serum of AA rats was increased significantly compared with the control group, whereas it was variably decreased in the GE group, which was consistent with synovial tissue (Fig. 3A, D). The changes in VEGF level further revealed the inhibitory effect of GE on angiogenesis in AA rats. In addition, it was also found that PTEN expression was decreased in synovial tissue of AA rats, while GE up-regulated PTEN expression (Fig. 3A, C). These results suggest that GE down-regulated angiogenic factors and exerted an anti-angiogenesis role in AA rats, which may be associated with PTEN expression.

**Establishment of inflammatory HUVECs model in vitro**

To confirm the role of PTEN in RA, an inflammation cell model of HUVECs induced by TNF-α and a co-culture cell model of FLSs-HUVECs were also established in addition to an AA rats model. First, the appropriate concentration of TNF-α (0, 1.25, 2.5, 5, 10, and 20 ng/mL) was screened to induce inflammation in HUVECs inflammatory model. CCK-8 results showed that the proliferation rate of HUVECs was the greatest at 10 ng/mL, while cell proliferation was instead inhibited at 20 ng/mL, which may be caused by cytotoxicity (Fig. 4A). Different concentrations of TNF-α showed different degrees of inhibition on PTEN expression, including 5 and 10 ng/mL (Fig. 4B). Based on the above results, 10 ng/mL was selected as the optimal stimulation concentration. HUVECs stimulated by different concentrations of FLSs-CM (0%, 5%, 25%, 50%, 75%, and 95%) were established to determine the optimal co-culture conditions. The proliferation rate of HUVECs stimulated by different concentrations of FLSs-CM is shown in Fig. 4C, 50% FLSs-CM promoted the proliferation of HUVECs significantly compared with the control group. Western blot showed that PTEN expression in 50–95% FLSs-CM was decreased compared with the control group (Fig. 4D). The above results support the selection of 50% FLSs-CM as the stimulation condition for the co-culture model.

Next, the two models were compared by VEGF secretion levels and PTEN protein and mRNA expression. As shown in Fig. 4E, F, PTEN protein expression and mRNA levels were decreased in TNF-α and FLSs-CM model compared with the control group. The VEGF secretion levels were also increased in both two models (Fig. 4G). Importantly, the P values between TNF-α and FLSs-CM groups were greater than 0.05, indicating that there was no significant difference in VEGF secretion and PTEN protein and mRNA expression between the two groups. The above results show that these two in vitro models simulated the environment of HUVECs in inflammatory and pro-angiogenic media, and the decrease of PTEN expression was consistent with that in the synovium of AA rats. For the consideration of experimental operability, the TNF-α (10 ng/mL) inflammation model was selected for subsequent experiments.

**PTEN over-expression inhibited proliferation, migration, and tube formation of HUVECs**

Our study showed that the expression of PTEN was decreased in the synovium of AA rats. To provide more evidence that PTEN is involved in RA angiogenesis, a PTEN lentiviral vector was used to over-express PTEN in HUVECs to assess the level of HUVECs proliferation, migration, and tube formation in vitro. Immunofluorescence and RT-qPCR analysis showed that the expression levels of PTEN protein and mRNA were significantly up-regulated after lentiviral vector infection, demonstrating successful infection (Fig. 5A, B).
The abnormal proliferation of HUVECs was the initial event of angiogenesis. Cell cycle results showed that the G1 phase decreased and the G2 and S phases increased after TNF-α stimulation; Lv-PTEN+ up-regulated the cells number of G1 phase cells and down-regulated the cells number of G2 and S phase (Fig. 5C). Similarly, Edu staining analysis showed that Lv-PTEN + inhibited the proliferation of HUVECs, which was determined by the reduction of green fluorescence representing proliferating cells (Fig. 5D). It was proved that HUVECs proliferation was promoted under inflammatory conditions, and PTEN over-expression inhibited proliferation. The migration of HUVECs as the basic behavior of angiogenesis was also examined by wound-healing and transwell assay. The wound-healing rate is directly proportional to the ability of cell migration. As shown in Fig. 5E, F, TNF-α stimulation significantly increased the healing rate and the number of migrated cells, while decreasing significantly in the Lv-PTEN+ group. As a classic model of angiogenesis in vitro, the HUVECs tube forming experiment is also analyzed. As shown in Fig. 5G, HUVECs in the TNF-α group were interconnected to form a complete lumen structure, whereas the lumen structure was damaged, the cell edge shrunk, and the number of branches decreased significantly after treatment with Lv-PTEN+. In conclusion,
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Fig. 4 Establishment and comparison of HUVECs inflammation model in vitro. 
A CCK-8 analysis of HUVECs' proliferation treated with different concentrations of TNF-α for 24 h (n = 6). B Western blot analysis of PTEN protein levels treated with different concentrations of TNF-α for 24 h (n = 3). C CCK-8 analysis of HUVECs proliferation treated with different concentrations of FLSs-CM for 24 h (n = 6). D Western blot analysis of PTEN protein levels treated with different concentrations of FLSs-CM for 24 h (n = 3). E Western blot analysis of PTEN protein levels in TNF-α inflammation model and FLSs-CM model (n = 3). F ELISA analysis of VEGF levels in TNF-α inflammation model and FLSs-CM model (n = 6). G RT-qPCR analysis of PTEN mRNA levels in TNF-α inflammation model and FLSs-CM model (n = 3). Data are represented as mean ± SD. #P < 0.05, ##P < 0.01 versus control group.
the over-expression of PTEN may be an important factor for inhibition of HUVECs angiogenesis.

**GE up-regulated PTEN to inhibit angiogenesis of HUVECs in vitro**

To further understand the therapeutic mechanism of GE and the potential involvement of PTEN, the in vitro model was used to explore the therapeutic effect of GE on HUVECs angiogenesis. Based on cell cycle analysis, the number of cells in the G1 phase decreased and G2 and S phases increased after PTEN inhibitor Bpv treatment. Different concentrations of GE down-regulated the number of G2 and S phase cells, indicating that GE had an obvious inhibitory effect on the proliferation of HUVECs (Fig. 6A). Edu staining analysis also showed that GE inhibited the proliferation of HUVECs by inhibiting PTEN. The healing
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rate and the number of migrating cells were decreased after GE treatment, indicating that GE inhibited HUVECs migration. In addition, the results of tube forming showed that GE also significantly inhibited the tube formation of HUVECs, which was manifested by the reduction of the number of tube and branching length (Fig. 6E). Our results indicate that the GE treatment attenuated the proliferation, migration, and tube formation of HUVECs, which may be related to the up-regulation of PTEN expression.

**GE regulated PTEN-PI3K-Akt signaling in HUVECs**

The above results show that over-expressed PTEN plays an important role in inhibiting angiogenesis. However, the
mechanism of PTEN in RA angiogenesis remains to be determined. Expression levels of PTEN-PI3K-Akt signal axis proteins, including PTEN, PI3K, p-PI3K, Akt, and p-Akt, were determined to evaluate the regulatory effect of GE on the signal axis. As shown in Fig. 7A–E, PTEN protein levels were decreased, while p-PI3K and p-Akt protein levels were increased in the TNF-α group compared with the control group. GE up-regulated PTEN protein level compared with TNF-α group, which was opposite to that of PTEN inhibitor Bpv group. In addition, GE exerted significant inhibitory effects on the expression of the proteins involved in the PI3K-AKT signal axis, as in the LY294002 group. Western blot results showed that GE up-regulated PTEN expression and inhibited the activation of PI3K-Akt signal.

To further confirm the associations among GE, PTEN, and PI3K-Akt signal axis, mRNA levels in HUVECs from each group were evaluated by RT-qPCR. As shown in Fig. 7F, PTEN mRNA level was significantly decreased in the TNF-α group, whereas PI3K and Akt mRNA levels were increased compared with the control group. GE up-regulated PTEN and induced significant suppression of PI3K-Akt signal axis-related mRNAs, which was consistent with the Western blot results. Our results suggested that GE inhibited the activation of PI3K-Akt signal axis by indirectly up-regulating PTEN.

**Discussion**

Chronic inflammation has always been considered as a significant pathological feature of RA and a therapeutic target of anti-arthritis drugs. However, studies have found that pro-inflammatory cytokines released by cells lining synovial tissue drive angiogenesis, which has attracted extensive attention as a fundamental event in pannus formation, whether it promotes FLSs tumor-like proliferation, inflammatory cell infiltration, or articular cartilage destruction (Cheung and McInnes 2017; Firestein and McInnes 2017). Angiogenesis in RA synovium can be defined by two phases: pre-vascular hyperinflammation stage and the prominent vascular stage. VECs are first activated, proliferate, and migrate under the stimulation of inflammatory mediators to form a capillary network to support the delivery of large amounts of oxygen and nutrients, allowing the recruitment of immune cells to the inflamed synovial tissue, where pro-inflammatory cytokines are further produced to participate in synovial proliferation. During the prominent vascular stage, nascent
microvessels, hyperplastic and hypertrophic synovial cells, inflammatory cells, and organized cellular form pannus, which maintains chronic inflammation, and even adhere to bone tissue to cause bone injury (Quiñonez-Flores et al. 2016; Littlejohn and Monrad 2018). Therefore, chronic inflammation and angiogenesis are considered to exist simultaneously and complement each other for the pathogenesis of RA. It is crucial to find ways to limit angiogenesis, based on the important role of angiogenesis in the inflammatory state of RA.

Appropriate animal models are the basis of experimental research and new drug development. The AA rat model shares many similar features with RA in clinical manifestations, pathology, and immunological changes, making it a more ideal animal model to study the pathological mechanisms and evaluate drug efficacy of RA (Bevaart et al. 2010; Choudhary et al. 2018). Considering the administration dose of GE in the treatment of AA rats and the previous study, the AA rat model was successfully established for in vivo evaluation in this study. The characteristics of the AA model were described in detail from the whole to the joint, and the evaluation indexes of the model were determined, including systemic evaluation, arthritis index, paw swelling level, and movement score. In addition, FLSs, as the main effector cell of RA joint synovium, contribute to and participate in synovium inflammation. VECs are not only an important component of vascular structure and angiogenesis, but also the material basis for the survival of FLSs, which is closely related to the occurrence and development of RA. Therefore, HUVECs were chosen for in vitro experiments, which are often used to perform vascular endothelial experiments because of stem cell potential. The cell co-culture model and the TNF-α stimulated cell model were established and compared to simulate synovial inflammation in vitro (Deng et al. 2021; Lee et al. 2012). Specifically, FLSs extracted from synovial tissue were co-cultured with HUVECs to simulate cell interaction in real synovial environment. On the other hand, TNF-α as a good stimulant induces HUVECs inflammation and vascular injury. TNF-α was found to be significantly elevated in RA, which not only increases the secretion of inflammatory cytokines and angiogenic mediators (such as IL-1 and VEGF), but also directly damages the function of endothelial cells or leads to vascular dysfunction, leading to vascular injury and local ischemia and hypoxia (Ogami et al. 2012; Mahdavi Sharif et al. 2020). Our experiments have demonstrated that FLSs and VECs are abnormally activated in the synovial tissue of AA rats, including abnormal proliferation and angiogenesis. The comparison of in vitro models showed that there was no significant difference between them in the detection of experimental related indexes (including cell proliferation, secretion of angiogenic factors, and PTEN expression). Finally, TNF-α was selected for the in vitro experiment, considering that FLSs cannot be guaranteed to be in the inflammatory state and the operability of the experiment in the in vitro co-culture experiment.

The angiogenesis process of RA is a programmed event, involving extracellular matrix (ECM) degradation, increased vascular permeability, endothelial cell proliferation, migration, sprouting, and remodeling. VECs, as the main constituent cells of vascular structure, whose biological behavior dominates the formation of new blood vessels. The vascular structure is obtained by the proliferation and remodeling of VECs, which is determined by differentiation into invasive tip cells and sprouting. The formation of capillary loops by sprouting tip cells to synthesize new basement membranes helps to maintain the structural and functional integrity of nascent blood vessels and ultimately the formation of new capillaries (Marrelli et al. 2011; Taylor and Sivakumar 2005; Volin and Koch 2011). There is evidence that inflammatory factors (IL-6, TNF-α, and MMP) in the synovium of RA patients are involved in VECs activation and recruitment of endothelial progenitor cells, thereby regulating the pathological activity of RA (Spinelli et al. 2013). Therefore, the ability of cell proliferation, migration, and tubulogenesis in vitro were comprehensively studied to evaluate the vascularization ability of HUVECs in an inflammatory environment and the treatment of GE.

As more and more angiogenic mediators that activate VECs are identified, such as growth factors, proteolytic enzymes, integrins, and adhesion molecules, researchers mostly focus on this (Bosisio et al. 2014; Kuczynski and Reynolds 2020). Previous studies by our group also focused on VEGF and found that VEGF binds to its surface receptors to activate downstream pathways of the cell and plays a role in mediating angiogenesis (Wang et al. 2021b; Sun et al. 2020). With the research of angiogenesis mechanism, researchers found that it is difficult to introduce the complex process of angiogenesis with a single signal, because it involves not only a single signal molecule, but also the cross-linking between multiple signals. Therefore, the discovery of novel approaches that target multiple cascades or select the upstream cascade of many angiogenic factors may provide a promising strategy for RA treatment. As early as the 1990s, researchers found the important role of genetic factors regulating angiogenic mediators in tumor angiogenesis. For example, Bouck first reported that the inactivation of the p53 gene by mutation or deletion downregulated the expression of thrombospondin, which induces angiogenesis (Bouck 1996; Sundaram et al. 2011; Ren et al. 2006). Thus, one of the main consequences of tumor suppressor gene inactivation is to promote tumor angiogenesis, which makes people re-recognize the importance of tumor suppressor genes (and oncogenes) in the study of angiogenesis. In this study, PTEN was proved to have a negative regulatory effect on HUVECs angiogenesis in an inflammatory environment, while up-regulation of PTEN expression may alleviate angiogenesis.
PI3K is an intracellular phosphatidylinositol kinase that phosphorylates PIP2 to produce PIP3 which in turn activates Akt (Chen et al. 2018). PTEN reverses this process by removing phosphate groups. PI3K-Akt signaling plays a role in a variety of cellular functions, including proliferation, survival, migration, invasion, and cell metabolism (Papa and Pandolfi 2019; Yi et al. 2020). Upstream components of the PI3K-Akt signaling pathway, such as PTEN and Ras, are commonly mutated in many human cancers, especially playing an important role in regulating normal vascularization and pathological angiogenesis. Direct evidence for the involvement of PI3K and Akt in regulating angiogenesis in vivo was observed by forced expression of PI3K and Akt by the RCAs retroviral vector system (Jiang et al. 2000). Over-expression of PI3K or Akt induces angiogenesis, whereas over-expression of PTEN inhibits angiogenesis in chicken embryos, indicating that PI3K-Akt signal is required for normal embryonic angiogenesis. In addition, PI3K is activated by growth factors and angiogenesis inducers, such as VEGF and Ang, activates Akt or other targets, and induces HIF-1 and VEGF expression to regulate angiogenesis (Karar and Maity 2011; Duan et al. 2019). PTEN-deficient endothelial cells showing increased excessive angiogenesis have been demonstrated in the vasculogenesis of tumors (Serra et al. 2015; Hafny-Rahbi et al. 2021). Akt regulates multiple downstream targets, including nitric oxide synthase (NOS), nuclear factor kappaB (NF-κB), glycogen synthase kinase 3 (GSK-3), and Jun N-terminal kinase (JNK). However, the specific target of Akt-induced angiogenesis remains to be determined. Based on the comprehensive research of RA disease and PTEN signal, we proposed for the first time that PTEN-PI3K-Akt signal is involved in the regulation mechanisms of RA angiogenesis. In the HUVECs model in vitro, we found that the expression levels of PTEN protein and mRNA were significantly decreased, and the expression levels of PI3K-Akt signal axis-related protein and mRNA were significantly increased after TNF-α stimulation. To further verify the involvement of the signal axis, a PTEN over-expression lentiviral vector was established. The results are consistent with our expectation that the over-expression of PTEN inhibited the proliferation, migration, and tubulogenesis of HUVECs in vitro. These findings support targeting the PTEN-PI3K-Akt signal as an effective strategy for the treatment of RA angiogenesis.

Pannus formation in RA depends on neovascularization, so inhibition of neovascularization can alleviate the progression of RA. Anti-angiogenic therapy may be another important measure following traditional drug treatments for RA. Inhibitors targeting the PI3K-Akt pathway have been developed to reduce VEGF secretion and angiogenesis as predicted. The traditional PI3K-Akt inhibitors LY294002 and wortmannin showed anti-angiogenic activity, but these inhibitors are not suitable for humans because of their toxicity and crossover inhibition of other lipid and protein kinases (Ouyang et al. 2005). The treatment of traditional Chinese medicine has attracted more and more attention as an effective treatment with the emergence of new technological approaches. GE, an iridoid glycoside obtained from Gardenia jasminoides Eills by modern extraction and separation technology of traditional Chinese medicine, is often used in the treatment of RA (Ran et al. 2021). In recent years, our team has performed a lot of work around the study of the therapeutic effect and mechanism of GE on RA. Previous studies have shown that GE can restore the balance of pro/anti-angiogenic factors and exert anti-angiogenic effects (Wang et al. 2021b). This study supports the role of GE in up-regulating PTEN expression to inhibit synovial angiogenesis. Especially in the in vitro model, the cellular biological function of HUVECs was significantly inhibited. Furthermore, we found that the anti-angiogenic effect of GE may be regulated by PTEN-PI3K-Akt signal. The results obtained by lentivirus vector and specific inhibitors are consistent with our expectation that regulating PTEN-PI3K-Akt signal can significantly reduce the angiogenesis of HUVECs, which is consistent with the therapeutic effect of GE (Fig. 8).

Conclusion

Angiogenesis is a prominent pathological feature of RA, which maintains pannus formation and runs through the whole disease. Our current results support that the low expression of PTEN in RA synovium is associated with excessive angiogenesis. GE effectively inhibited secondary inflammation and angiogenesis in AA rats and up-regulated the expression of PTEN. In addition, GE treatment attenuated the proliferation, migration, and tubulogenesis of HUVECs, which was concerned with up-regulating PTEN and inhibiting the activation of PI3K-Akt pathway. The present findings offer a new option for therapeutic intervention of RA. Further studies are needed to determine the specific mechanism of GE up-regulating PTEN expression, and this part of the experiment is in progress.
The anti-angiogenesis mechanism of Geniposide on rheumatoid arthritis is related to the...

Author contributions
Participated in research design: YB and HW. Conducted experiments: YB, RD, and YW. Performed data analysis: YB, RD, and YW. Wrote or contributed to the writing of the manuscript: YB.

Funding
This work was supported by grants from the National Natural Science Foundation of China (No 81874360 and No 81073122) and the major projects of Natural Science Research in Anhui Universities (KJ2021ZD0060).

Data availability
The data generated in this study have been analyzed and included in this article, and unprocessed data will be available on request.

Declarations
Conflict of interest
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics approval
All animal studies designed in this article have been approved by the experimental animal ethics committee of Anhui University of Traditional Chinese Medicine (No. ahucm-rates-2021049). It is considered that this study meets the requirements of animal ethics in animal species selection, quantity, feeding, and modeling.

References
Alam J, Jantan I, Bukhari SNA (2017) Rheumatoid arthritis: recent advances on its etiology, role of cytokines and pharmacotherapy. Biomed Pharmacother 92:615–633
Balogh E, Biniecka M, Fearon U, Veale DJ, Szekanecz Z (2019) Angiogenesis in inflammatory arthritis. Isr Med Assoc J 21(5):345–352
Bevaart L, Vervoordeldonk MJ, Tak PP (2010) Evaluation of therapeutic targets in animal models of arthritis: how does it relate to rheumatoid arthritis? Arthritis Rheum 62(8):2192–2205
Bosisio D, Salvi V, Gagliostro V, Sozzani S (2014) Angiogenic and anti-angiogenic chemokines. Chem Immunol Allergy 99:89–104
Bouck N (1996) P53 and angiogenesis. Biochim Biophys Acta 1287(1):63–66
Chen CY, Chen J, He L, Stiles BL (2018) PTEN: tumor suppressor and metabolic regulator. Front Endocrinol (Lausanne) 9:338
Cheung TT, McInnes IB (2017) Future therapeutic targets in rheumatoid arthritis? Semin Immunopathol 39(4):487–500
Choudhary N, Bhatt LK, Prabhavalkar KS (2018) Experimental animal models for rheumatoid arthritis. Immunopharmacol Immunotoxicol 40(3):193–200
Deng R, Bu Y, Li F, Wu H, Wang Y, Wei W (2021) The interplay between fibroblast-like synovial and vascular endothelial cells leads to angiogenesis via the sphingosine-1-phosphate-induced RhoA-F-Actin and Ras-Erk1/2 pathways and the intervention of geniposide. Phytother Res 35(9):5305–5317
Duan MX, Zhou H, Wu QG et al (2019) Andrographolide protects against HG-induced inflammation, apoptosis, migration, and impairment of angiogenesis via PI3K/AKT-eNOS signalling in HUVECs. Mediators Inflamm 2019:6168340
El Hafny-Rahbi B, Brodaczewska K, Collet G et al (2021) Tumour angiogenesis normalized by myo-inositol trisphosphate alleviates hypoxia in the microenvironment and promotes antitumor immune response. J Cell Mol Med 25(7):3284–3299
Elshabrawy HA, Chen Z, Volin MV, Essani AB et al (2018) IL-11 facilitates a novel connection between RA joint fibroblasts and endothelial cells. Angiogenesis 21(2):215–228
Firestein GS, McInnes IB (2017) Immunopathogenesis of rheumatoid arthritis. Immunity 46(2):183–196
Jiang BH, Zheng JZ, Aoki M, Vogt PK (2000) Phosphatidylinositol 3-kinase signaling mediates angiogenesis and expression of vascular endothelial growth factor in endothelial cells. Proc Natl Acad Sci USA 97(4):1749–1753
Karar J, Maitty A (2011) PI3K/AKT/mTOR pathway in angiogenesis. Front Mol Neurosci 4:51
Kuczynski EA, Reynolds AR (2020) Vessel co-option and resistance to anti-angiogenic therapy. Angiogenesis 23(1):55–74
Lee AS, Kim JS, Lee YJ, Kang DG, Lee HS (2012) Future therapeutic targets in rheumatoid arthritis. Semin Immunopathol 39(4):487–500
Choudhary N, Bhatt LK, Prabhavalkar KS (2018) Experimental animal models for rheumatoid arthritis. Immunopharmacol Immunotoxicol 40(3):193–200
Deng R, Bu Y, Li F, Wu H, Wang Y, Wei W (2021) The interplay between fibroblast-like synovial and vascular endothelial cells leads to angiogenesis via the sphingosine-1-phosphate-induced RhoA-F-Actin and Ras-Erk1/2 pathways and the intervention of geniposide. Phytother Res 35(9):5305–5317
Duan MX, Zhou H, Wu QG et al (2019) Andrographolide protects against HG-induced inflammation, apoptosis, migration, and impairment of angiogenesis via PI3K/AKT-eNOS signalling in HUVECs. Mediators Inflamm 2019:6168340
El Hafny-Rahbi B, Brodaczewska K, Collet G et al (2021) Tumour angiogenesis normalized by myo-inositol trisphosphate alleviates hypoxia in the microenvironment and promotes antitumor immune response. J Cell Mol Med 25(7):3284–3299
Elshabrawy HA, Chen Z, Volin MV, Essani AB et al (2018) IL-11 facilitates a novel connection between RA joint fibroblasts and endothelial cells. Angiogenesis 21(2):215–228
Firestein GS, McInnes IB (2017) Immunopathogenesis of rheumatoid arthritis. Immunity 46(2):183–196
Jiang BH, Zheng JZ, Aoki M, Vogt PK (2000) Phosphatidylinositol 3-kinase signaling mediates angiogenesis and expression of vascular endothelial growth factor in endothelial cells. Proc Natl Acad Sci USA 97(4):1749–1753
Karar J, Maitty A (2011) PI3K/AKT/mTOR pathway in angiogenesis. Front Mol Neurosci 4:51
Kuczynski EA, Reynolds AR (2020) Vessel co-option and resistance to anti-angiogenic therapy. Angiogenesis 23(1):55–74
Lee AS, Kim JS, Lee YJ, Kang DG, Lee HS (2012) Anti-TNF-α antibody activates hypoxia in the microenvironment and promotes antitumor immune response. J Cell Mol Med 25(7):3284–3299
Elshabrawy HA, Chen Z, Volin MV, Essani AB et al (2018) IL-11 facilitates a novel connection between RA joint fibroblasts and endothelial cells. Angiogenesis 21(2):215–228
Firestein GS, McInnes IB (2017) Immunopathogenesis of rheumatoid arthritis. Immunity 46(2):183–196
Jiang BH, Zheng JZ, Aoki M, Vogt PK (2000) Phosphatidylinositol 3-kinase signaling mediates angiogenesis and expression of vascular endothelial growth factor in endothelial cells. Proc Natl Acad Sci USA 97(4):1749–1753
Karar J, Maitty A (2011) PI3K/AKT/mTOR pathway in angiogenesis. Front Mol Neurosci 4:51
Kuczynski EA, Reynolds AR (2020) Vessel co-option and resistance to anti-angiogenic therapy. Angiogenesis 23(1):55–74
Lee AS, Kim JS, Lee YJ, Kang DG, Lee HS (2012) Anti-TNF-α activity of Portulaca oleracea in vascular endothelial cells. Int J Mol Sci 13(5):5628–5644
Li Q, Zhao H, Chen W, Huang P, Bi J (2019) Human keratinocyte-derived microvesicle miRNA-21 promotes skin wound healing in diabetic rats through facilitating fibroblast function and angiogenesis. J Med Invest 66(4):250–256
Li Q, Zhao H, Chen W, Huang P, Bi J (2019) Human keratinocyte-derived microvesicle miRNA-21 promotes skin wound healing in diabetic rats through facilitating fibroblast function and angiogenesis. J Med Invest 66(4):250–256
Littlejohn EA, Monrad SU (2018) Early diagnosis and treatment of chronic inflammation? Autoimmun Rev 10(10):595–598
Naito H, Iba T, Takakura N (2020) Mechanisms of new blood-vessel formation and proliferative heterogeneity of endothelial cells. Int Immunol 32(5):295–305
Ogami K, Yamaguchi R, Imoto S et al (2012) Computational gene network analysis reveals TNF-induced angiogenesis. BMC Syst Biol 6(Suppl 2):S12
Ouyang W, Li J, Shi X, Costa M, Huang C (2005) Essential role of PI-3K, ERKs and calcium signal pathways in nickel-induced VEGF expression. Mol Cell Biochem 279(1–2):35–43
Papa A, Pandolfi PP (2019) The PI-3K/AKT pathway in cancer. Biomolecules 9(4):153
Quiñonez-Flores CM, González-Chávez SA, Pacheco-Tena C (2016) PI3K/AKT pathway in degenerated discs. Mol Med Rep 13(5):5628–5644
Portulaca oleracea in vascular endothelial cells. Int J Mol Med 30(8):4347–4362
Ran D, Hong W, Gan Y, Mengdie W (2021) Properties and molecular mechanisms underlying geniposide-mediated therapeutic effects in chronic inflammatory diseases. J Ethnopharmacol 273:113958
Ren B, Yee KO, Lawler J, Khosravi-Far R (2006) Regulation of tumor angiogenesis by thrombospondin-1. Biochim Biophys Acta 1765(2):178–188
Serra H, Chivite I, Angulo-Urarte A et al (2015) PTEN mediates Notch-dependent stalk cell arrest in angiogenesis. Nat Commun 6:7935
Spinelli FR, Metere A, Barbati C et al (2013) Effect of therapeutic inhibition of TNF on circulating endothelial progenitor cells in patients with rheumatoid arthritis. Mediators Inflamm 2013:537539
Sun M, Deng R, Wang Y et al (2020) Sphingosine kinase 1/sphingosine 1-phosphate/sphingosine 1-phosphate receptor 1 pathway: a novel target of geniposide to inhibit angiogenesis. Life Sci 256:117988
Sundaram P, Hultine S, Smith LM et al (2011) p53-responsive miR-194 inhibits thrombospondin-1 and promotes angiogenesis in colon cancers. Cancer Res 71(24):7490–7501
Taylor PC, Sivakumar B (2005) Hypoxia and angiogenesis in rheumatoid arthritis. Curr Opin Rheumatol 17(3):293–298
Volin MV, Koch AE (2011) Interleukin-18: a mediator of inflammation and angiogenesis in rheumatoid arthritis. J Interferon Cytokine Res 31(10):745–751
Wang Y, Wu H, Deng R (2021a) Angiogenesis as a potential treatment strategy for rheumatoid arthritis. Eur J Pharmacol 910:174500
Wang Y, Wu H, Deng R et al (2021b) Geniposide downregulates the VEGF/SphK1/S1P pathway and alleviates angiogenesis in rheumatoid arthritis in vivo and in vitro. Phytother Res 35(8):4347–4362
Xue L, Huang J, Zhang T et al (2018) PTEN inhibition enhances angiogenesis in an in vitro model of ischemic injury by promoting Akt phosphorylation and subsequent hypoxia inducible factor-1α upregulation. Metab Brain Dis 33(5):1679–1688
Yi J, Zhu J, Wu J, Thompson CB, Jiang X (2020) Oncogenic activation of PI3K-AKT-mTOR signaling suppresses ferroptosis via SREBP-mediated lipogenesis. Proc Natl Acad Sci USA 117(49):31189–31197
Zhang H, Wang P, Zhang X, Zhao W, Ren H, Hu Z (2020) SDF1/CXCR4 axis facilitates the angiogenesis via activating the PI3K/AKT pathway in degenerated discs. Mol Med Rep 22(5):4163–4172

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