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Echinocystic acid activates PPARγ to alleviate mannan-induced psoriasis and psoriatic arthritis in mice

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Abstract
Previous studies have shown that echinocystic acid (EA) can reduce arthritis and skin damage, but the role of EA in psoriatic arthritis is unclear. This study aims to prove the role of EA in psoriatic arthritis, which was induced by intraperitoneal injection of mannan in C57BL/6J mice. The mice were divided into a control group, mannan group, mannan + EA (low-dose) group, and mannan + EA (high-dose) group. Joint tissue damage was scored, and pathological changes in joint tissue and ear skin damage were examined by HE staining. Pathway enrichment of EA drug targets was performed through the target enrichment website, and the mRNA and protein expression levels of pathway-related proteins in joint tissues and ears were verified using the PCR and western blot. The results show that injection of mannan into mice resulted in joint inflammatory infiltration and tissue damage, hyperkeratosis, and acanthosis of the ear skin, while these symptoms were alleviated after high-dose EA treatment. Pathway enrichment analysis showed that the EA drug treatment target is concentrated on the PPAR pathway. The mRNA and protein results showed that the mRNA and protein expression levels of peroxisome proliferator-activated receptor γ (PPARγ) in the joint tissues and ears of mice with psoriatic arthritis decreased, and the expression of PPARγ was activated after high-dose EA treatment. In conclusion, EA increases PPARγ expression and reduces joint and skin damage in mice with psoriatic arthritis.

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Introduction

Psoriatic arthritis is a chronic inflammatory arthritis caused by psoriasis. It usually affects the skin, causing symptoms of psoriasis, which are characterized by hyperplasia of the epidermis with red spots and silvery scales. It can also affect the joints, causing pain, swelling, and stiffness. Psoriatic arthritis is a complex disease, and its exact cause is not fully understood, but genetic factors, the immune system, and environmental factors may all play a role.[1]

Echinocystic acid (EA), a pentacyclic triterpene extracted from herbs such as Codonopsis pilosula and Acacia quinata, has many positive effects. As a natural compound, EA has few side effects and is easily accessible. Therefore, EA has been widely used to treat various diseases. Recently, many studies have shown that EA has anti-inflammatory, antioxidant, and anti-apoptotic effects[2] and can reduce synovial hyperplasia in arthritis and reduce the content of pro-inflammatory factors.[3] It can improve the symptoms of atopic dermatitis, reduce epidermal and dermal thickness, prevent immune cell infiltration, and restore skin barrier function.[4] Based on the above studies, it is speculated that EA may play a protective role and alleviate the symptoms of psoriatic arthritis.

One of the three known PPAR receptor isoforms is PPAR-γ. Research has demonstrated that the PPARγ pathway plays a role in the development of psoriasis and that increasing the PPAR-γ pathway can reduce imiquimod-induced keratinocyte hyperproliferation.[5] PPARγ agonists have anti-inflammatory properties that prevent bone and joint degeneration in animal models of osteoarthritis.[6]

This study showed that EA reduced the symptoms of psoriasis and psoriatic arthritis and the EA targets were enriched in the PPAR pathway. In vivo tests showed that EA triggered the expression of PPARγ in psoriatic mice. This study provides both a reference for psoriatic arthritis research and possible therapeutic drugs for its treatment.

Methods

Animals

During the experiment, specific pathogen-free C57BL/6J mice (6–8 weeks old) were kept in a setting with consistent humidity and temperature and unrestricted access to food and drink. The mice were divided into four groups: control group (n=6), mannan group (n=6), mannan+EA (10 mg/kg) group (n=6), and mannan+EA (20 mg/kg) group (n=6). The mice in the mannan group were intraperitoneally injected with 20 mg of mannan in 200 μL phosphate-buffered saline (PBS) on days 0, 4, and 8. The mice in the mannan+EA group were gavaged with 10 mg/kg or 20 mg/kg of EA starting 3 days before the injection of mannan for 15 consecutive days. The paws with arthritis had the following scores: 0 for redness or swelling; 1 for mild swelling or redness with acceptable mobility; 2 for moderate redness or swelling with acceptable mobility; 3 for moderate redness or swelling with poor mobility; and 4 for severe redness or swelling with loss of mobility.

Histological analysis

Animal joint and ear tissue samples were collected, and the tissue samples were treated with paraformaldehyde to fix proteins and cellular structures. The samples were placed in a gradient concentration of alcohol to dehydrate the tissues and then placed in xylene to make the tissues transparent. The joint tissue was embedded in paraffin and cut into 4 μM slices using a microtome. The tissue sections were then dewaxed and hydrated, and the tissue was stained according to the HE staining procedure. Finally, the histopathological changes were observed under a microscope.

Bioinformatics analysis

The PharmMapper website (http://www.lilab-ecust.cn/pharmmapper/) was used to collect EA targets and the Uniprot website (https://www.uniprot.org/) was used to transform gene names, yielding 430 targets. Pathway enrichment of targets was done using Metascape (https://metascape.org/gp/index.html#/main/step1) and Enrichr (https://maayanlab.cloud/Enrichr/) websites.

Quantitative real-time PCR analysis

The TRIzol reagent was used to extract total RNA from the joints and ears of mice. cDNA was produced by reverse transcription of mRNA. Using the SYBR Green PCR Master Mix kit and real-time PCR detection equipment, quantitative real-time PCR was carried out, with GAPDH serving as an internal reference. The $2^{-\Delta\Delta Ct}$ technique was used to evaluate the relative expression of mRNA. The sequence is as follows: PPARγ, F: 5'-CAAGAATACCAAAGTGCGATCAA-3', R: 5'-GAGCTGGGTCTTTTCAGAATAATAAG-3'. GAPDH, F: 5'-TTGTTGCCATCAACGACCCC-3', R: 5'-GCCGTTGAATTTGCCGTGAG-3'.

Western blot

Mouse joint and ear samples were quickly frozen with liquid nitrogen and ground into powder form. Then, RIPA lysis buffer was added, lysed on ice, and the supernatant was obtained by centrifugation. The protein bands in the samples were separated by the SDS-PAGE gel electrophoresis system and were transferred to PVDF membranes. The PVDF membranes were incubated with 5% skim milk at room temperature for 1 hour, washed with TBST, and incubated with PPARγ antibody (Abcam, UK, ab272718) at 4°C overnight. After washing, they were incubated with secondary antibodies at room temperature for 1 hour and washed again with TBST. After visualization with the ECL reagent, the membranes were scanned using the Bio-Rad gel imaging system, and protein expression was analyzed using the ImageJ software.

Statistical analysis

GraphPad Prism 10.0 was used to analyze all of the data, and the results were presented as mean ± SD. Multiple

groups were compared using one-way analysis of variance, and the significance of the differences was assessed using the Dunnett's test.

Results

EA alleviates symptoms of psoriatic arthritis in mice

The changes in the paws of mice in each group were scored (Figure 1A) and photographed (Figure 1B). The paws of mice in the control group showed no changes and were scored as 0. The paws of mice in the mannan group became red and swollen, and the joint scores increased significantly. Treatment with EA (10 mg/kg) did not significantly improve the redness and swelling of the paws induced by mannan, nor did it reduce its joint scores, but EA (20 mg/kg) treatment alleviated the redness and swelling of the paws induced by mannan and reduced the joint scores. In addition, HE staining was used to observe the histopathological changes of the ankle joints of mice in each group (Figure 1C). The mice in the mannan group and the mannan+EA (10 mg/kg) group showed synovial hyperplasia and inflammatory infiltration, and the above symptoms were alleviated in the mice in the mannan+EA (20 mg/kg) group, indicating that EA (20 mg/kg) can alleviate the symptoms of psoriatic arthritis in mice.

EA attenuates psoriasis skin damage in mice

The ear skin of mice in each group was examined and photographed (Figure 2A), followed by HE staining (Figure 2B). The ear skin of mice in the control group was normal, while the ears of mice in the mannan group and mannan+EA (10 mg/kg) group showed hyperkeratosis and acanthosis, and the above symptoms of mice were alleviated in the mannan+EA (20 mg/kg) group, indicating that EA (20 mg/kg) can alleviate the symptoms of psoriasis skin damage in mice.

Bioinformatics analysis showed that EA targets were enriched in the PPAR pathway

The drug targets of EA were collected through the PharmMapper website, and pathway enrichment of the targets was performed through the Metascape and Enrichr websites, respectively. The bar chart of enrichment analysis (Figure 3A) and the diagram of clustering network (Figures 3B and 3C) were obtained from the Metascape website, and the results showed that the genes were enriched in the PPAR signaling pathway. In addition, the diagram of enrichment analysis in the Enrichr website also showed that the genes were enriched in the PPAR signaling pathway (Figure 3D).

EA increases PPARγ expression in skin and joint tissues of mice with psoriatic arthritis

To confirm whether EA can regulate PPARγ in vivo, the expression of PPARγ in ear and joint tissues was detected by PCR and WB. The results showed that the mRNA expression level of PPARγ in the ear and joint tissues of psoriatic arthritis mice was decreased, while the mRNA expression

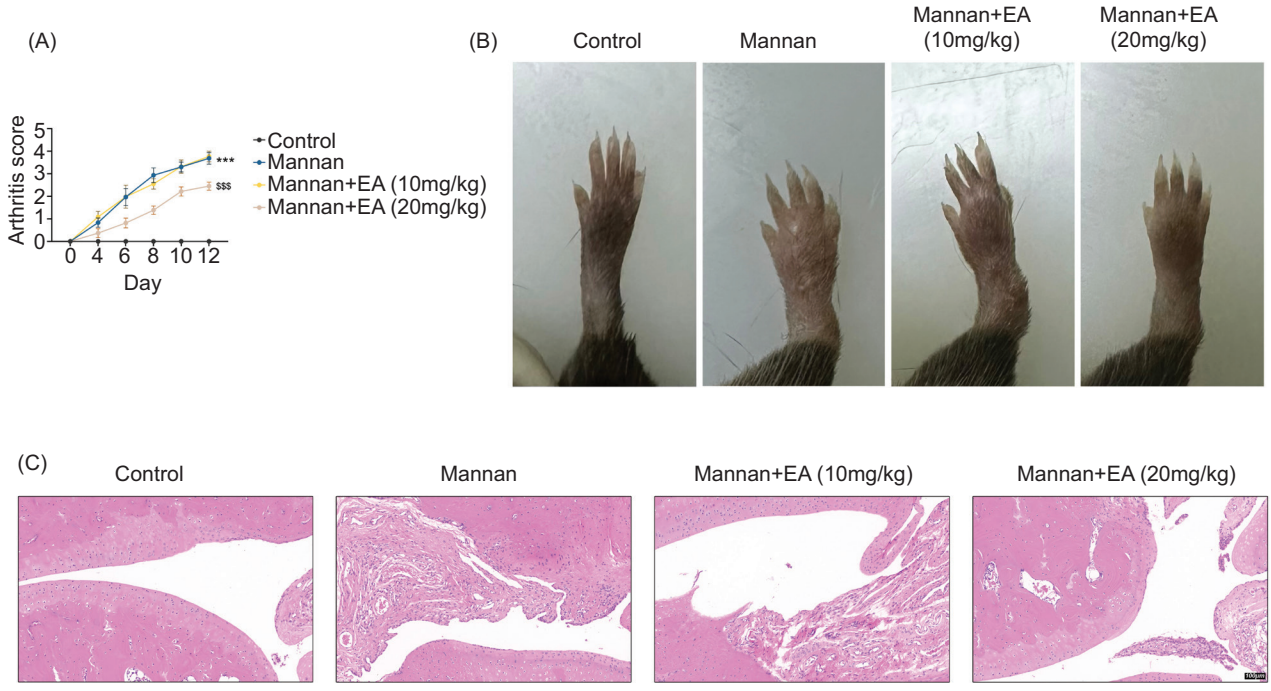

Figure 1 EA alleviates psoriatic arthritis symptoms in mice. (A) Arthritis scores of mice in each group within 12 days. (B) The degree of swelling and redness of the paws of mice in each group. (C) HE staining to examine the pathological changes of joint tissues of mice in each group.

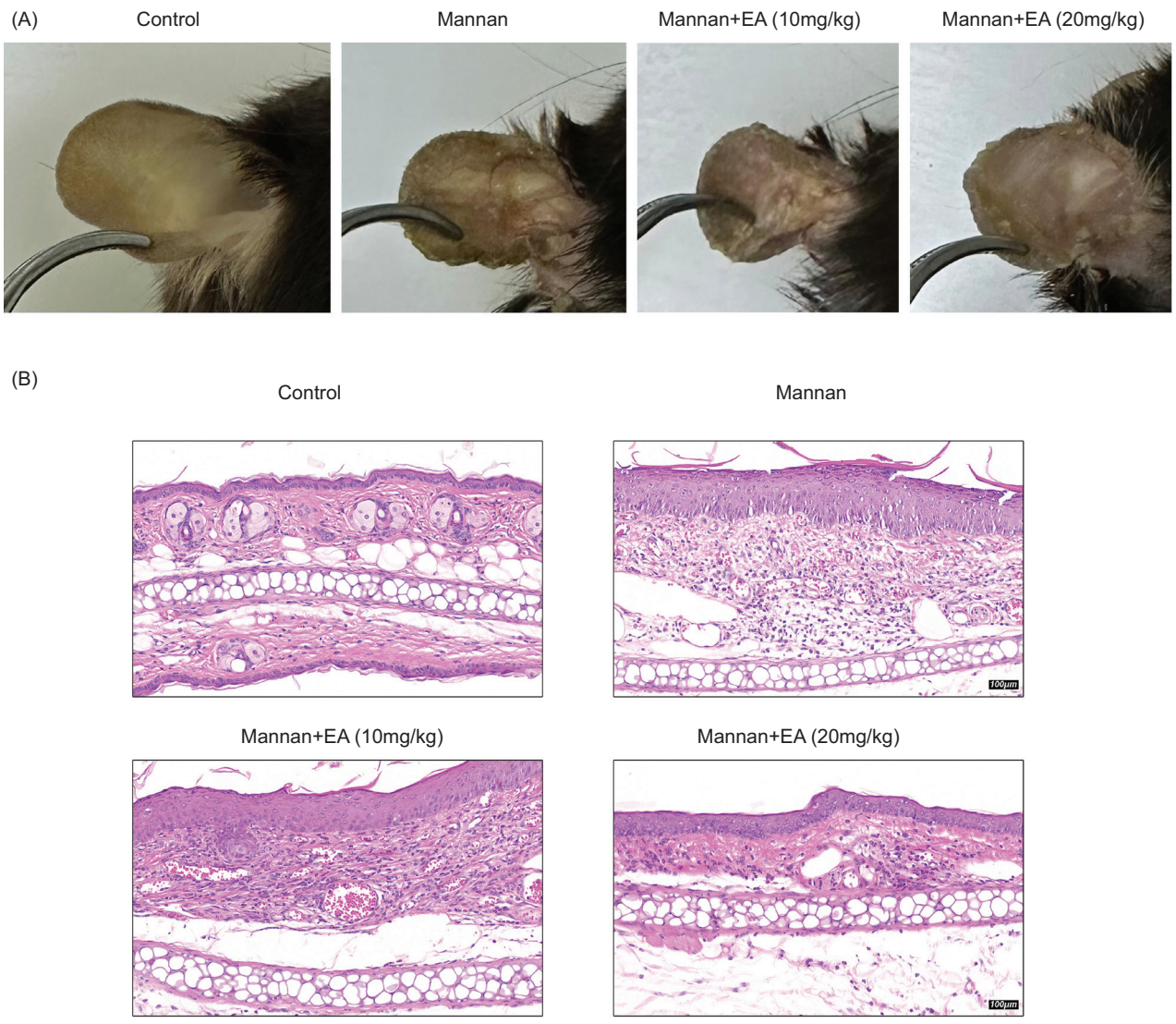

Figure 2 EA attenuates psoriasis skin damage in mice. (A) Take photos of the ear skin damage of mice in each group. (B) HE staining to examine the pathological changes in the ears of mice in each group.

level of PPARγ in mice treated with EA (20 mg/kg) was increased (Figure 4A). Similarly, the protein expression level of PPARγ in the ear and joint tissues of mice with psoriatic arthritis was decreased, while the level of protein expression of PPARγ in mice treated with EA (20 mg/kg) was increased (Figure 4B). This indicates that EA can increase the expression of PPARγ in the skin and joint tissues of mice with psoriatic arthritis.

Discussion

It is generally accepted that psoriasis is an immune-mediated skin condition that affects about one-third of the people who develop psoriatic arthritis.[7] In mice with psoriatic arthritis, EA reduced joint swelling and ear skin. In addition, it triggered the expression of PPARγ in skin and joint tissues. Thus, these data imply that EA inhibits psoriasis-related skin and joint lesions by activating PPARγ.

EA is a pentacyclic triterpene derived from the inflorescence of the leguminous plant, Albizia julibrissin Durazz. It has anti-inflammatory, antioxidant, anti-apoptotic, and anti-tumor properties. EA alleviates hypoxic-ischemic brain neuronal damage and apoptosis in newborn mice, inhibits oxidative stress and brain atrophy, and plays a neuroprotective role in brain injury.[8] In obese rats that have a high-fat diet, EA can lower triglyceride levels, which may help in preventing obesity, diabetes, and nonalcoholic steatohepatitis.[9] By controlling inflammation and apoptosis, EA can reduce sepsis-related acute kidney injury in mice and be a natural medication for the condition.[10] In addition to inhibiting the formation of osteoclasts and the expression of marker proteins linked to osteoclastogenesis, EA also molecularly suppressed RANKL-induced NF-κB activation and ERK phosphorylation in bone marrow macrophages and in the future may be used as a medication to treat disorders involving osteoclasts[11] EA successfully reduced the inflammatory response that IL-1β generated in osteoarthritis

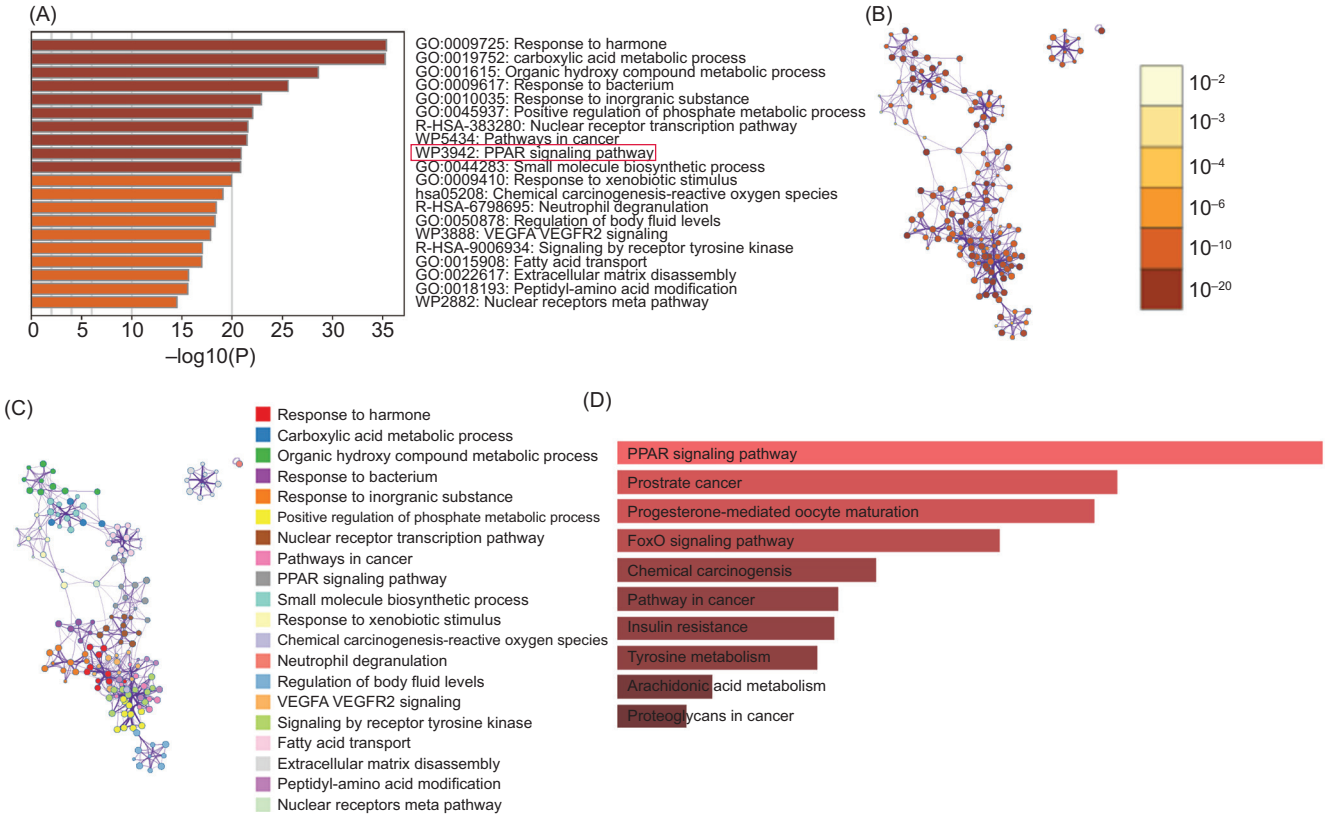

Figure 3 Bioinformatics analysis showed that EA targets were enriched in the PPAR pathway. (A) Bar chart of pathway enrichment of EA targets on the Metascape website. (B) Clustering network diagram of pathway enrichment of EA targets on the Metascape website (colored by cluster ID). (C) Clustering network diagram of pathway enrichment of EA targets on the Metascape website (colored by p-value). (D) Bar chart of pathway enrichment of EA targets using the Enrichr website.

chondrocytes, indicating that it could be a useful treatment for osteoarthritis[12] EA treatment can prevent the decrease of femoral maximum stress and Young's modulus in osteoporotic rats induced by ovariectomy, restore the levels of bone metabolism biomarkers in rats, and reduce the levels of IL-1β and TNF-α in rat serum, which may be a potential drug for anti-osteoporosis.[13] The above studies all show that EA has a protective effect with regard to bone damage. For the first time, this study demonstrated that EA can lessen joint damage because of psoriatic arthritis by reducing the redness and swelling of the paws of the mice afflicted by psoriatic arthritis, as well as inflammatory infiltration and minor cartilage erosion of joints.

Three PPAR nuclear receptors function as ligand-activated transcription factors, including the peroxisome proliferator-activated receptor (PPAR-γ). The activation of PPAR-γ modulates the immune response, encourages cell differentiation, and decreases the rate of proliferation. According to certain research, psoriasis patients have a lower level of PPAR-γ. Furthermore, they also suggested using PPAR-γ agonists as an adjuvant psoriasis treatment.[14] Studies conducted on mice suggest that either PPAR-γ overexpression or agonist-induced activation may result in various skin benefits. They normalize the terminal differentiation of epidermal keratinocytes and reduce their rate of proliferation. The rate of proliferation of epidermal keratinocytes is reduced when PPAR-γ agonists are administered to animals and cultured cells. Baicalin can significantly improve skin lesions in mice with psoriasis and reduce the levels of inflammatory factors by activating PPARγ.[15] In addition, studies have shown that activation of PPARγ can alleviate the progression of osteoarthritis. Pretreatment with Angelica sinensis polysaccharide can scavenge ROS, enhance mitochondrial metabolism, boost chondrocyte viability, reduce apoptosis, and activate PPARγ in rat chondrocytes treated with tert butyl hydroperoxide.[16] Therapy using the PPARgamma agonist is a promising treatment for psoriatic arthritis, as evidenced by the use of PPARγ agonists by certain researchers in patients with psoriatic arthritis.[17] In this study, EA targets were enriched by two target enrichment websites (Metascape and Enrichr), and the results showed that EA targets were mainly enriched in the PPAR signaling pathway. Animal in vivo experiments also confirmed that PPARγ expression was significantly reduced in the joint tissues and ears of mice with psoriatic arthritis, while the mRNA and protein expression levels of PPARγ were significantly increased after EA treatment, indicating that EA activates PPARγ in psoriatic arthritis.

In conclusion, this study is the first to demonstrate that EA activates PPARγ in psoriatic arthritis and reduces joint damage and skin lesions. This study provides a reference

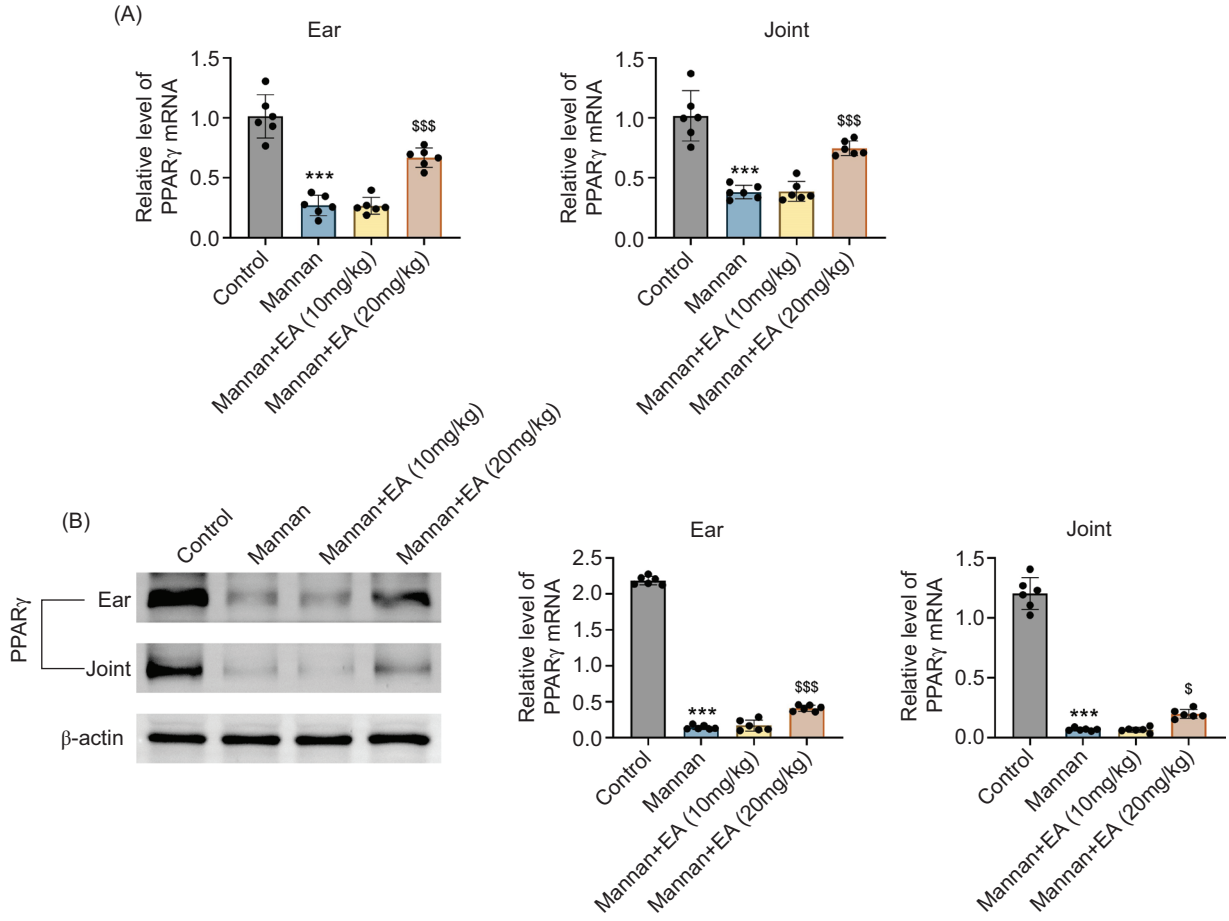

Figure 4 EA increases PPARγ expression in skin and joint tissues of psoriatic arthritis mice. (A) PCR detection of PPARγ mRNA expression levels in joint tissues and ears. (B) Western blot detection of PPARγ protein expression levels in joint tissues and ears.

for candidate drugs for the treatment of psoriatic arthritis. Because of funding constraints, this study is not very comprehensive. We did not perform in vitro experiments to verify the role of EA, nor did we study other pathways enriched in EA targets. In the future, we will continue to study the role of EA in psoriatic arthritis.

Acknowledgments

Not applicable.

Authors Contribution

Chengwei Yu designed and conducted the study; Chengwei Yu, Huiming Wu, Dongrui Zhao, and Huajie Shi supervised the data collection; Chengwei Yu, Huiming Wu, Dongrui Zhao, and Huajie Shi analyzed the data; Chengwei Yu, Huiming Wu, Dongrui Zhao, and Huajie Shi interpreted the data; Chengwei Yu and Huajie Shi prepared and reviewed the manuscript for publication. All authors have read and approved the manuscript.

Conflicts of Interest

The authors state that there are no conflicts of interest to disclose.

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Ethics Approval

Ethical approval was obtained from Experimental Animal Ethics Committee of Wenzhou Medical University (Approval no. wydw2024-0386).

Consent to Participate Statement

Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

Data Availability

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

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