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Effects of Cyclopamine on the Viability of Articular Chondrocytes in Rats with Adjuvant Arthritis *In Vitro* and Part Mechanisms

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Abstract

The aim of the present study was to investigate the effect of cyclopamine, a hedgehog signaling pathway inhibitor, on Adjuvant Arthritis (AA), rat articular chondrocyte viability and part mechanisms *in vitro*. In this study, an AA rat model was established by Freund's Complete Adjuvant (FCA), and the Arthritis Index (AI), secondary paw swelling degree and HE staining was used to evaluate whether the model was successfully established. Chondrocytes of the ankle joint of AA rats were cultured and identified. Cyclopamine (0, 0.03, 0.1, 0.3, 1, 3, 10 and 30 mg/L) was administered to determine chondrocyte viability. Chondrocyte apoptosis was detected by Annexin V-FITC/PI double dye. The expression of hedgehog signaling pathway-related proteins Shh, Ptch1 and Gli1 in chondrocytes was detected by western blotting. The results showed that AA was successfully induced by FCA, since the AI of AA rats and secondary paw swelling degree increased, the cartilage tissue of the rats' ankle joint was damaged, and the chondrocytes were successfully cultured *in vitro* following the identification of toluidine blue and type II collagen. Cyclopamine (0.03, 0.1, 0.3, 1, 3, 10 and 30 mg/L) could increase the viability of chondrocytes *in vitro* and reduce the apoptotic rate of chondrocytes. As compared with the control group, different doses of cyclopamine (0.3, 3 and 10 mg/L) significantly decreased the expression of Shh, Ptch1 and Gli1 proteins in AA chondrocytes. Therefore, in the present study, an AA rat model was successfully established, and cyclopamine inhibited the viability and apoptosis of chondrocytes, an effect that may be associated with the inhibition of the chondrocyte hedgehog signaling pathway.

Keywords: Adjuvant arthritis; Articular chondrocytes; Cyclopamine; Hedgehog; Viability; Western blotting

Introduction

Synovial cell viability and inflammatory cell infiltration are common in patients with Rheumatic Arthritis (RA), RA is an autoimmune disease, which can damage subsynovial cartilage and bone tissue, and cause joint deformity and dysfunction. The main cause of disability is the destruction of articular cartilage and bone tissue. Therefore, the key to treatment is to effectively prevent articular cartilage and bone tissue damage [1]. The hedgehog signaling pathway is a conserved evolution from lower animals such as fruit flies to higher animals as human, which regulates the embryonic tissue differentiation and tumor formation [2]. In recent years, the role of hedgehog signaling in regulating bone and cartilage development and growth has attracted considerable attention. Studies have confirmed that, following the activation of the hedgehog signaling pathway, specific gene blocking or silencing was obvious in the articular cartilage cells of patients with osteoarthritis, which could effectively alleviate osteoarthritis in these patients and provide a new approach for the prevention and treatment of joint disease [3,4]. Currently, only a few studies have been conducted on the effect of hedgehog pathway inhibitor cyclopamine on articular chondrocytes *in vitro*. This study used Adjuvant Arthritis (AA) models in Wistar rats to determine the influence of the hedgehog pathway inhibitor cyclopamine on articular chondrocyte activation in AA rats *in vitro*, expecting to provide a new scientific basis for RA pathogenesis.

Materials and Methods

Experimental animals

Twenty male Wistar rats (6-8 weeks) weighing (140 ± 10) g were purchased from Biological Engineering Co., LTD. Nanjing Junke (Animal Approval Number 001). Rats were raised in temperature (22.5 ± 1.5)°C- and humidity (60.0 ± 2.0)% controlled rooms for 1 week. Rats had free access to standard food and tap water. The light cycle is 14 hours of light and 10 hours of darkness. The animals were used in the experiments according to the guidelines of the Animal Experimental Ethics Committee of Anhui Medical University (reference no. 2017001; Hefei, China). Twenty Wistar rats were randomly divided into two groups; the control and model groups. 100 μL Freund's Complete Adjuvant (FCA) was injected into the right hind toe of rats for 28 days (It's a one-time injection and then fed for 28 days). The control group rats were injected with the same amount of physiological saline.

Main reagents and instruments

The following reagents and instruments were used: Tuberculin derivation protein (Beijing Xiangrui Biological Products Co., Ltd., Beijing, China); Anhydrous lanolin (Xianbao Co., Ltd., Changchun, China); FCA, cyclopamine, anti-Smo, anti-Ptch1 and anti-Gli1 polyclonal antibodies (Merck KGaA, Darmstadt, Germany); total protein extraction kit (Invent Biotechnologies, Inc., Plymouth, MN, USA); nitrocellulose membrane (EMD Millipore, Billerica, MA, USA). Enzyme-linked immunosorbent assay plate reader (EON, USA); YLS-

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7C Toes Volume Measurement Instrument (Beijing ZhongshiShidi Technology Co., Ltd., Beijing, China).

Paw swelling degree and arthritis index (AI) score

The parameters of non-injected swelling (left hind paw of rats) and polyarthritis index of the AA rats were evaluated every 4 days, from day 12 to 28 following injection. The degree of AI was determined using a scoring protocol [5,6] where by severity was scored on a scale of 0–4, where 0=absent, 1=minimal, 2=mild, 3=moderate, and 4=severe. With the development of rheumatoid arthritis, the degree of secondary joint swelling is an important index to evaluate rheumatoid arthritis. The left hind paw volume (ankle joint) was tested using the YLS-7C Toes Volume Measurement Instrument. Paw swelling degree (Δ mL)=paw volume after injection-paw volume before injection.

HE staining

Ankle joints of rats were harvested, fixed in 4% paraformaldehyde solution for 24 hours at 20°C, and immersed in 10% with EDTA decalcification solution for 8 weeks. Ankle joints were cut into 4 μm sized slices and mounted on a glass slide. The sections were stained with HE at 20°C (hematoxylin stain for 5 min and eosin stain for 30 s. Images of the stained tissue were obtained using light microscopy.

Culture of chondrocytes

Chondrocytes were isolated from the model group, as previously described [7]. In brief, the trypsin -II collagenase combined separation method was used: the surface cartilage of Wistar rat ankle joint was isolated aseptically; pancreatic enzymes digested for 30 min at 37˚C; 0.2% type II collagenase digested for 3 hour; strained through a 200 mesh filter; centrifuged (800xgx10 min at 20˚C); washed with PBS (5minx3). Cells were resuspended and plated in DMEM (St Louis, MO, USA) with 10% fetal bovine serum (St Louis, MO, USA) and then cultured at 37˚C and 5% CO_2 until 70% confluence in a 15 cm plate (Figure 1).

Identification of chondrocytes

Collagen type II immunocytochemistry:Cells were washed with PBS for 3 times, fixed with 4% paraformaldehyde for 1 hour at room temperature and were permeabilized with 0.1% Triton X-100. The slides were blocked with Immunol Staining Blocking Buffer (Beyotime, Shanghai, China) at room temperature for 1 hour and then were incubated with Anti-Collagen II (1 : 200, ab34712, Abcam) overnight at 4°C. After washing with PBS, the slides were incubated for 40 min at 37°C with a secondary antibody (1 : 400, 43413, Sigma). Finally, the slides were washed twice with PBS. Images of the stained cells were obtained using light microscope (Leica, Germany).Toluidine blue staining: cells were washed with PBS for 3 times, fixed with 4% paramethylal at room temperature for 30 min, stained with 1% toluidine blue at room temperature for 2 hour, rinsed with anhydrous ethanol for 2 times, and sealed with neutral gum after drying.

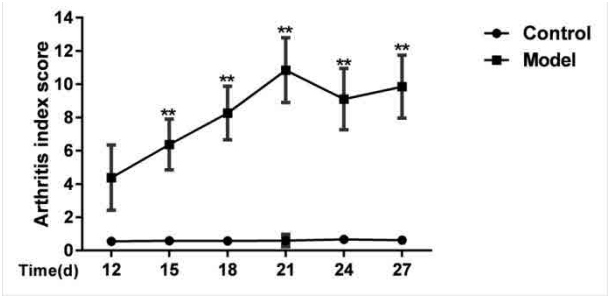

Figure 1: AI score of AA rats at the indicated time points ($\bar{x}$ ± s, n=10). **p<0.01 *vs.* normal group. AI: Arthritis Index; AA: Adjuvant Arthritis.

Chondrocyte viability assay

Chondrocytes were subcultured in 96-well plates at a density $5x10^7$/L. Following treatment with cyclopamine (0.03, 0.1, 0.3, 1, 3, 10 and 30 mg/L) for 44 h at 37°C with 5% CO_2, 10 μL of 5 mg/mL MTT solution was added to each well and incubated for 4 hour at 37°C. Subsequently, the supernatant was removed, and the formazan crystals were dissolved in 150 μL DMSO. 96-well plates were shaked evenly resulting in complete dissolution of formazan. Absorbance was measured at 570 nm on an enzyme standard instrument (Figure 2).

Apoptotic rate of articular chondrocytes detected in AA rats

The apoptotic rate of chondrocytes was detected by flow cytometry using an Annexin V-FITC/PI staining kit (BestBio, Shanghai, China). Chondrocytes were seeded in 6-well plates at a density of $5x10^7$/mL, and treated with cyclopamine (0.3, 3 and 10 mg/L). After 48 hour, the cells were harvested. Following the protocol: Cells were washed twice in cold PBS and 400 μL Annexin V binding buffer, 5 μL FITC and 10 μL PI were then added for 5 min at 4˚C in the dark. Finally, cells were analyzed by flow cytometry (FACSCalibur, BD Biosciences). Images were obtained using FlowJo 7.6 software (USA). Each of the experiments was repeated 3 times, n=3.

Western blotting detected the protein expression of Shh, Gli1 and Ptch1 in chondrocytes

Cells were seeded in 6-well plates and treated with cyclopamine (0.3, 3 and 10 mg/L) at 37°C with 5% CO_2 for 24 hours. Cells were washed 3 times with cold PBS, then suspended in 200 μL of RIPA Lysis buffer (containing 1 mmol/L protease inhibitor, PMSF) (Beyotime, Shanghai, China) for 1 min. After centrifugation at 13,000×g for 15 min at 4°C. The protein concentration was determined by BCA assay kit. An equal quantity of proteins (50 μg) were separated using 10% and 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to PVDF membranes. After the membranes were blocked with 5% skim milk for 1 hour at room temperature, they were incubated with the anti-Shh (1 : 1,000, S8321, Sigma), anti-Gli (1 : 1,500, SAB4501219, Sigma), anti-Ptch (1 : 1,500, SAB2500835, Sigma), anti-GAPDH (1 : 1,500, SAB3500350, Sigma) primary antibodies overnight at 4°C. The next day, membranes were washed 3 times with TBST for 5 min and incubated with horseradish peroxidase-conjugated sheep anti-rabbit IgG secondary anti-bodies (1 : 1,000, 43413, Sigma) for 1 hour at room temperature. They were then washed 3 times with TBST for 5 min. Finally, the immuno-reactive bands were detected with an Enhanced Chemiluminescent (ECL) reagent (BOSTER, Wuhan, China) using gel analysis software BandScan 5.0 and analysed by Image Pro Plus software. Each of the experiments was repeated 3 times, n=3.

Statistical analysis

Data are expressed as the mean ± standard deviation using SPSS 22.0 statistical software (IBM Corp., Armonk, NY, USA) to determine significant differences between the groups. Statistical analysis was performed using Student's t-test or one–way ANOVA followed by

SNK-q test, where appropriate. P<0.05 was considered to indicate a statistically significant difference.

Results

Secondary AI score and paw swelling degree

Paw arthritis severity was evaluated by the AI in the non-injection side (secondary) of rats. The results showed that, following injection for 12, 15, 18 and 21 days, the AI of AA rats had gradually increased, as compared with the control group (p<0.05). The paw swelling degree was also found to have gradually increased (p<0.05).

Morphological structure of cartilage tissue

HE staining revealed a thin layer of synovial tissue on the surface of chondrocytes in the normal control group, and showed that chondrocytes had gradually grown from the margin to the center, revealing a group of homologous cells. Abnormal hyperplasia of the synovial tissue was observed in the ankle joint of the model group, invading the cartilage tissue, and chondrocytes were disordered (Figure 3).

Chondrocyte identification and culture

Primary chondrocytes were spherical, and many spindle and polygonal chondrocytes were shown to be attached to the wall at ~10 hours in, completely adhering to the wall in ~3 days. Approximately a week later, the cells were connected into sheets and could be sub-cultured. Toluidine blue staining showed that the cytoplasm of chondrocytes was light blue and their nucleus blue. A few heterochromatic particles were identified in the cytoplasm

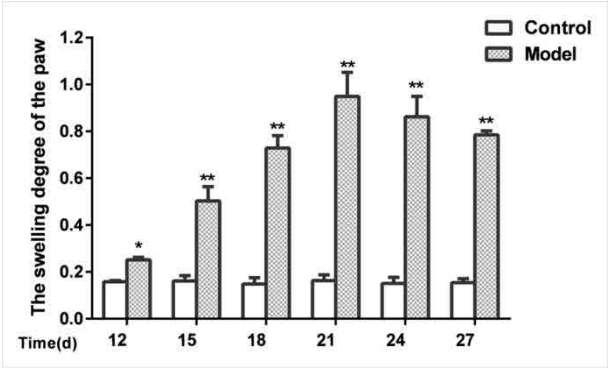

Figure 2: Swelling degree of the secondary paw in AA rats at the different indicated time points. *p<0.05 and **p<0.01 *vs.* normal group. AA: Adjuvant Arthritis.

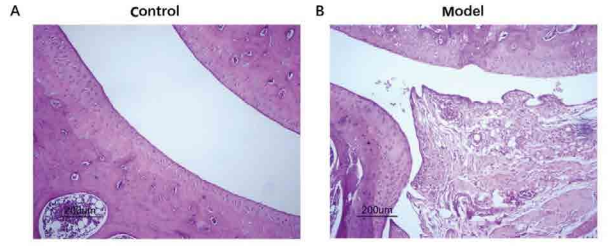

Figure 3: HE staining (Magnification 100X). (A) Control group. (B) Model group, synovial tissue infiltration.

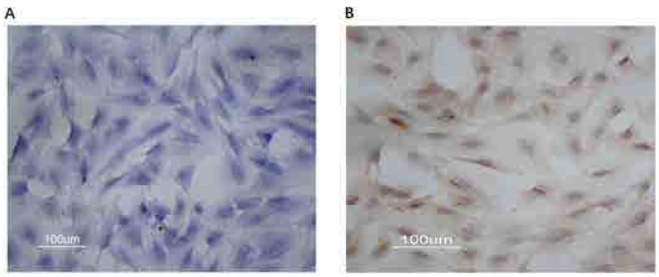

Figure 4: Identification of chondrocytes. (A) Positive toluidine blue staining. (B) Positive collagen type Ⅱ immunohistochemical staining positive (Magnification 200X).

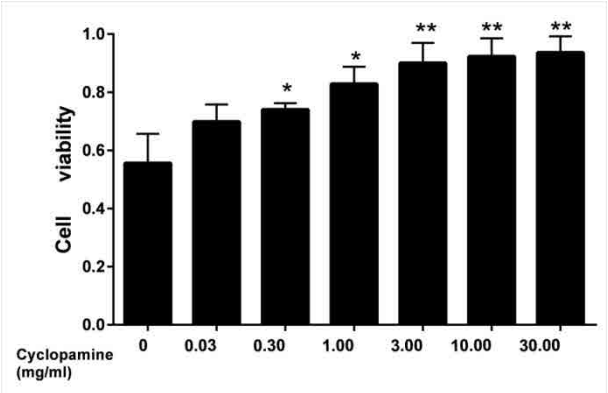

Figure 5: Effect of different concentrations of cyclopamine on chondrocyte viability ($\bar{x}$ ± s, n=10).

(Figure 4). Type II collagen immunohistochemical staining showed that the cytoplasm of chondrocytes exhibited a brown-yellow type II collagen expression (Figure 5).

AA rat chondrocyte viability

As shown in Figure 5, chondrocyteviability was significantly increased following treatment with cyclopamine (0.03, 0.3, 1, 3, 10 and 30 mg/L), and had dase dependent. Considering that 0.03 mg/L cyclopamine had no obvious effect on chondrocytes, the final concentrations of cyclopamine used in subsequent experiments were 0.3, 3 and 10 mg/L.

Apoptotic rate of chondrocytes in AA rats

Annexin V-FITC/PI staining was used to detect chondrocyte apoptosis as shown in Figure 6, the apoptotic rate of chondrocytes gradually decreased with the increase in cyclopamine concentration (p<0.05). The rates of early apoptosis in chondrocytes were (5.6 ± 0.5)%, (4.4 ± 0.6)% and (3.1 ± 0.4)%, respectively, significantly lower than those in the model group (10.6 ± 0.8)% (p<0.05).

Effect of cyclopamine on the expression of hedgehog pathway-related proteins in chondrocytes

As shown in Figure 7, the expression of hedgehog pathway-related proteins Shh, Gli1 and Ptch1 were detected by western blotting in the chondrocytes of AA rats. Compared with the control group, the expression of these proteins was significantly increased in chondrocytes,

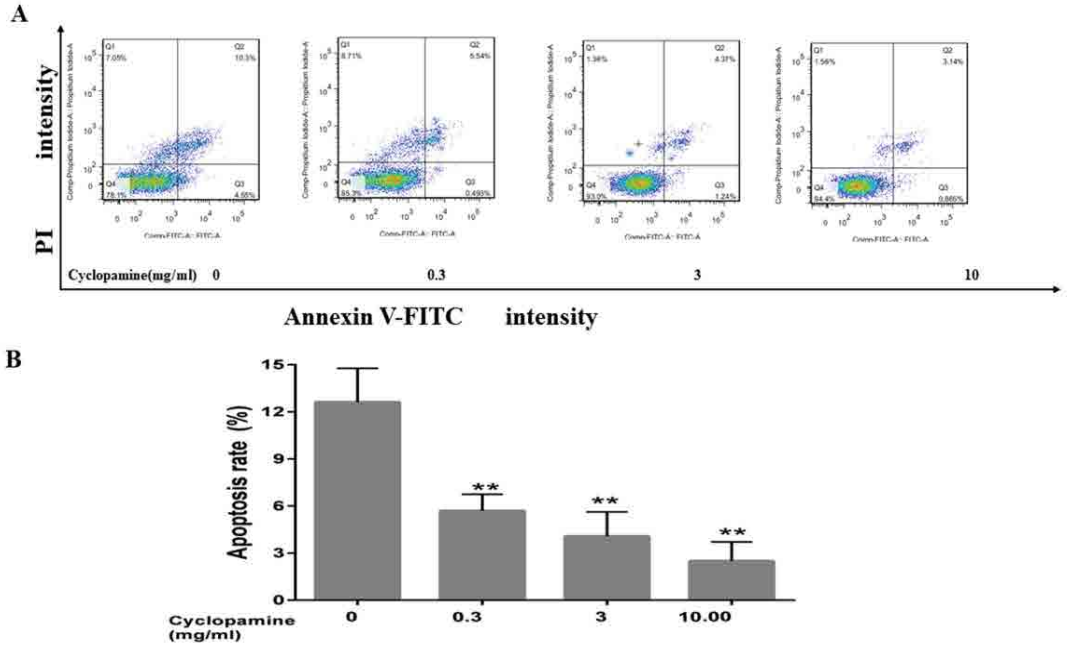

Figure 6: Apoptotic rate of articular chondrocytes detected in AA rats. (A) Representative images of flow cytometric analysis by Annexin V-FITC/PI staining. (B) Quantitative determination of apoptotic rate ($\bar{x}$ ± s, n=3). *p<0.05 and **p<0.01 *vs*. model group. AA: Adjuvant Arthritis.

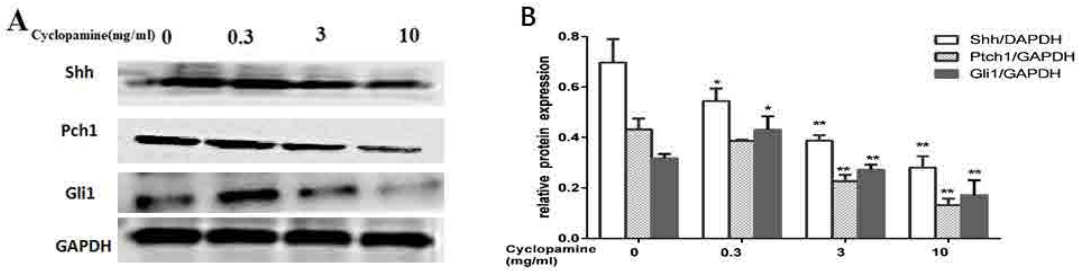

Figure 7: Effect of cyclopamine on the expression of hedgehog pathway-related proteins in chondrocytes. (A) Representative western blotting images; (B) Quantitative analysis of protein expression ($\bar{x}$ ± s, n=3). *p<0.05 and **p<0.01 *vs*. model group.

following treatment with different concentrations of cyclopamine (0, 0.3, 3 and 10 mg/L) for 24 hours.

Discussion

RA is an autoimmune disease caused by abnormal viability in the synovial tissue, progressive destruction of the cartilage, and bone and joint dysfunction [1,8]. In the present study, an AA model of Wistar rats was established by FCA, which is a classic animal model of RA pathogenesis [5,9]. Compared with normal rats, the AA model rats exhibited a quick onset, with the hind legs affected before the forelimbs, ankle suffered most easily, in addition to the injection paw, secondary parapodum paws underwent inflammatory changes [10]. In order to evaluate secondary paw arthritis, semi-quantitative scoring was used to score secondary side inflammation and calculate the AI and joint swelling degree in this study [11,12]. The research showed that secondary AI and secondary joint swelling were obvious in AA rats, indicating that the AA rat model was successfully established. HE staining showed synovial tissue hyperplasia in the ankle joint of the model group, invasion of cartilage tissue, and disordered arrangement of chondrocytes, which indicated the successful establishment of the model.

The main pathological features and clinical manifestations of RA are cartilage, bone and joint destruction, and its pathogenesis is not completely clear [13]. Articular cartilage tissue covers the surface of joint bone, mainly including chondrocytes and extracellular matrix [14]. Chondrocytes have an active metabolism, which is crucial to chondrogenesis, metabolism and repair [15]. During RA, chondrocyte apoptosis leads to decreased cartilage density, impaired matrix metabolism balance, and articular cartilage damage [7]. Type II collagen is an important component of cartilage matrix. Toluidine blue staining results showed that the nuclei of chondrocytes stained blue and the cytoplasm light blue, confirming that the culture of AA rat chondrocytes was successful.

Currently, there is a lack of clinically specific drugs for RA treatment. Cyclopamine is an isosteroid alkaloid isolated from veratrol plants, which can inhibit the growth of tumor cells [4]. In the present study, MTT assay showed that cyclopamine could increase the viability of joint chondrocytes in AA rats in a dose-dependent manner *in vitro*.

To study this effect, we used Annexin V, which can be sensitive to detecting cell surface phosphatidylserine of the probe, the principle of PI staining can observe the apoptosis [16], the results showed that chondrocyte apoptosis gradually decreased with the increase in cyclopamine concentration, and the result was significant, as compared with the model group. It has been suggested that cyclopamine administration could effectively reduce apoptosis in AA articular chondrocytes *in vitro*. Limitedly, our experiments only demonstrated the effect of cyclopamine on chondrocytes in AA rats, and no study was conducted on normal chondrocytes. We will use of sham chondrocytes constitutes an important control for further comparisons of the effect of cyclopamine. The experiment is now under way.

Studies have shown that the activated hedgehog signaling pathway is involved in bone and joint destruction during the progression of osteoarthritis [17]. The hedgehog signaling pathway has four main aspects: Hedgehog signaling molecules, Dhh, Shh and Ihh; transmembrane receptors, Smo and Ptch; Glis family of nuclear transcription factors, and downstream target genes [18,19]. In general, Ptch inhibits Smo activity following its interaction with Smo, leading to the inhibition of hedgehog signaling and decreased cell viability. In inflammationdiseases, Hh protein binds to Ptch, weakening Ptch's inhibition of Smo, activating the downstream nuclear transcription factor Glis family, thus inducing the expression of target genes and aggravating inflammation. As an important pathway regulating the growth and development of bone and cartilage [20,21], the role of abnormal hedgehog signaling has been linked to the development of bone and joint [22]. In this study, western blotting results showed that, as compared with the control group, the protein level of Shh, Ptchl and Glil in articular chondrocytes from AA rats significantly decreased following treatment with cyclopamine (0.3, 3 and 10 mg/L) for 24 hour, suggesting that the hedgehog signaling pathway in AA rat chondrocytes had been abnormally activated. Cyclopaminedrug delivery *in vitro* could lead to a significant reduction in the protein expression pathway, which might be closely associated with the inhibition of viability and decline of apoptosis in articular chondrocytes of AA rats following treatment with cyclopamine.

Conclusion

In conclusion, in the present study, an AA rat model was successfully established by FCA, and the articular chondrocytes of AA rats were isolated, cultured and identified. The cyclopamine pathway inhibitor cyclopamine could significantly improve the viability of articular chondrocytes from AA rats and inhibit their apoptosis. This effect may be associated with the inhibition of the abnormal activation of the hedgehog signaling pathway.

Funding

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Availability of Data and Materials

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

Authors' Contributions

Xianbin Song and Xiaoyu Chen designed the study. Bao Zhang, Yanping Huang,Ye Zhang and Meimei Liu performed the experiments. Taorong Wang and Xiaoyu Chen analyzed the data and prepared the manuscript. All authors reviewed the results and approved the final version of the manuscript.

Ethics Approval and Consent to Participate

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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References

1. Adams SB, Reilly RM, Huebner JL, Kraus VB, Nettles DL, et al. (2017) Time-Dependent Effects on Synovial Fluid Composition During the Acute Phase of Human Intra-articular Ankle Fracture. Foot Ankle Int 38: 1055-1063.
2. Biehs B, Dijkgraaf GJP, Piskol R, Alicke B, Boumahdi S, et al. (2018) A cell identity switch allows residual BCC to survive Hedgehog pathway inhibition. Nature 562: 429-433.
3. Li R, Cai L, Ding J, Hu CM, Wu TN, et al. (2015) Inhibition of hedgehog signal pathway by cyclopamine attenuates inflammation and articular cartilage damage in rats with adjuvant-induced arthritis. J Pharm Pharmacol 67: 963-971.
4. Ali SA, Al-Jazrawe M, Ma H, Whetstone H, Poon R, et al. (2016) Regulation of Cholesterol Homeostasis by Hedgehog Signaling in Osteoarthritic Cartilage. Arthritis Rheumatol 68: 127-137.
5. Gupta S, Mishra KP, Singh SB, Ganju L (2018) Inhibitory effects of andrographolide on activated macrophages and adjuvant-induced arthritis. Inflammopharmacology 26: 447-456.
6. Wang T, Wang G, Zhang Y, Zhang J, Cao W, et al. (2018) Effect of lentivirus-mediated overexpression or silencing of MnSOD on apoptosis of resveratrol-treated fibroblast-like synoviocytes in rheumatoid arthritis. Eur J Pharmacol 844: 65-72.
7. Zhang J, Song X, Cao W, Lu J, Wang X, et al. (2016) Autophagy and mitochondrial dysfunction in adjuvant-arthritis rats treatment with resveratrol. Sci Rep 6: 32928.
8. Mao YM, Zhao CN, Leng J, Leng RX, Ye DQ, et al. (2018) Interleukin-13: A promising therapeutic target for autoimmune disease. Cytokine Growth Factor Rev.
9. Wang X, He X, Zhang CF, Guo CR, Wang CZ, et al. (2017) Anti-arthritic effect of berberine on adjuvant-induced rheumatoid arthritis in rats. Biomed Pharmacother 89: 887-893.
10. Leng P, Li D, Sun Y, Wang Y, Zhang H, et al. (2018) Effects of human cyclooxygenase-2 gene silencing on synovial cells of rheumatoid arthritis mediated by lentivirus. Artif Cells Nanomed Biotechnol 1-7.
11. Zijlstra TR, Bernelot Moens HJ, Bukhari MA (2002) The rheumatoid arthritis articular damage score: first steps in developing a clinical index of long term damage in RA. Ann Rheum Dis 61: 20-23.
12. Anderson JK, Zimmerman L, Caplan L, Michaud K. (2011) Measures of rheumatoid arthritis disease activity: Patient (PtGA) and Provider (PrGA) Global Assessment of Disease Activity, Disease Activity Score (DAS) and Disease Activity Score with 28-Joint Counts (DAS28), Simplified Disease Activity Index (SDAI), Clinical Disease Activity Index (CDAI), Patient Activity Score (PAS) and Patient Activity Score-II (PASII), Routine Assessment of Patient Index Data (RAPID), Rheumatoid Arthritis Disease Activity Index (RADAI) and Rheumatoid Arthritis Disease Activity Index-5 (RADAI-5), Chronic Arthritis Systemic Index (CASI), Patient-Based Disease Activity Score With ESR (PDAS1) and Patient-Based Disease Activity Score without ESR (PDAS2), and Mean Overall Index for Rheumatoid Arthritis (MOI-RA). Arthritis Care Res (Hoboken) 63 Suppl 11: S14-36.
13. Scott DL, Wolfe F, Huizinga TW (2010) Rheumatoid arthritis. Lancet 376: 1094-1108.
14. Lebre MC, Jongbloed SL, Tas SW, Smeets TJ, McInnes IB, et al. (2008) Rheumatoid arthritis synovium contains two subsets of CD83-DC-LAMP-dendritic cells with distinct cytokine profiles. Am J Pathol 172: 940-950.
15. Otero M, Goldring MB (2007) Cells of the synovium in rheumatoid arthritis. Chondrocytes. Arthritis Res Ther 9: 220.

16. Du K, Hyun J, Premont RT, Choi SS, Michelotti GA, et al. (2018) Hedgehog-YAP Signaling Pathway Regulates Glutaminolysis to Control Activation of Hepatic Stellate Cells. Gastroenterology 154: 1465-1479.

17. Thiruvalluvan M, Barghouth PG, Tsur A, Broday L, Oviedo NJ, et al. (2018) SUMOylation controls stem cell proliferation and regional cell death through Hedgehog signaling in planarians. Cell Mol Life Sci 75: 1285-1301.

18. Bangs F, Anderson KV (2017) Primary Cilia and Mammalian Hedgehog Signaling. Cold Spring Harb Perspect Biol 9.

19. Maurya DK, Bohm S, Alenius M (2017) Hedgehog signaling regulates ciliary localization of mouse odorant receptors. Proc Natl Acad Sci USA 114: 9386-9394.

20. Ehlen HW, Buelens LA, Vortkamp A (2006) Hedgehog signaling in skeletal development. Birth Defects Res C Embryo Today 78: 267-279.

21. Bechtold TE, Saunders C, Decker RS, Um HB, Cottingham N, et al. (2016) Osteophyte formation and matrix mineralization in a TMJ osteoarthritis mouse model are associated with ectopic hedgehog signaling. Matrix Biol 52-54: 339-354.

22. Song M, Han L, Chen FF, Wang D, Wang F, et al. (2018) Adipocyte-Derived Exosomes Carrying Sonic Hedgehog Mediate M1 Macrophage Polarization-Induced Insulin Resistance via Ptch and PI3K Pathways. Cell Physiol Biochem 48: 1416-1432.