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DPSCs Attenuate Experimental Progressive TMJ Arthritis by Inhibiting the STAT1 Pathway

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Abstract

Severe inflammation, progressive cartilage, and bone destruction are typical pathologic changes in temporomandibular joint (TMJ) arthritis and lead to great difficulty for treatment. However, current therapy is inefficient to improve degenerative changes in progressive TMJ arthritis. This study investigated the therapeutic effects of human dental pulp stem cells (DPSCs) on severe inflammatory TMJ diseases. Progressive TMJ arthritis in rats was induced by intra-articular injection of complete Freund's adjuvant and monosodium iodoacetate. DPSCs were injected into the articular cavity to treat rat TMJ arthritis, with normal saline injection as control. Measurement of head withdrawal threshold, micro–computed tomography scanning, and histologic staining were applied to evaluate the severity of TMJ arthritis. Results showed that local injection of DPSCs in rats with TMJ arthritis relieved hyperalgesia and synovial inflammation, attenuated cartilage matrix degradation, and induced bone regeneration. Inflammatory factors TNF-α and IFN-γ were elevated in progressive TMJ arthritis and partially decreased by local injection of DPSCs. MMP3 and MMP13 were elevated in the arthritis + normal saline group and decreased in the arthritis + DPSCs group, which indicated amelioration of matrix degradation. The isolated primary synoviocytes were cocultured with DPSCs after inflammatory factors stimulated to explore the possible biological mechanisms. The expression of MMP3 and MMP13 in synoviocytes was elevated after TNF-α and IFN-γ stimulation and partially reversed by DPSC treatment in the in vitro study. The signal transducer and activator of transcription 1 (STAT1) was activated by inflammatory stimulation and suppressed by DPSC coculture. The upregulation of MMP3 and MMP13 triggered by inflammation was blocked by STAT1-specific inhibitor, suggesting that STAT1 regulated the expression of MMP3 and MMP13. In conclusion, this study demonstrated the possible therapeutic effects of local injection of DPSCs on progressive TMJ arthritis by inhibiting the expression of MMP3 and MMP13 through the STAT1 pathway.

Keywords: temporomandibular joint disorders, mesenchymal stem cells, arthritis, synovium membrane, cartilage, matrix metalloproteinases

Introduction

Inflammation has an intensive relationship to the occurrence and progression of temporomandibular joint (TMJ) disorders (Kellesarian et al. 2016). Sustained synovitis, cartilage destruction, subchondral bone remodeling, and pain often occur together in patients with TMJ disorders (Stegenga 2001). The current clinical therapies for inflammatory TMJ diseases focus on oral or intra-articular injection drugs, such as nonsteroidal anti-inflammatory drugs, corticosteroid, glucosamine, and hyaluronate (Liu et al. 2018). These drugs effectively alleviate pain but show minimal improvement in cartilage and bone loss (Sun et al. 2018). Therefore, an efficient method for tissue repair and regeneration needs to be established.

Previous studies suggested that activated immune inflammatory response may trigger the cascade downstream reaction of matrix degradation in TMJ (Monasterio et al. 2018). The elevated expression of proinflammatory factors, such as interferon γ (IFN-γ) and tumor necrosis factor α (TNF-α), has been found in synovial fluid from patients with TMJ osteoarthritis (OA; Vernal et al. 2008). These cytokines secreted by synoviocytes influence the function of chondrocytes and aggravate subsequent cartilage degradation (Huh et al. 2015; Kellesarian et al. 2016).

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Matrix metalloproteinases (MMPs) are a family of proteinases that directly degrade the extracellular matrix and influence cartilage remodeling (Gross and Lapiere 1962). Cartilage degradation and bone deterioration in inflammatory TMJ diseases are related to the level of MMPs (Jiao et al. 2014). Overexpressed MMPs were detected in synovial fluid from patients with progressive inflammatory TMJ diseases (Luo et al. 2015). Cartilage matrix regeneration and bone degradation are the key challenges of clinical treatment for arthritis (Lu et al. 2015). Thus, regulating the expression of MMPs could be a promising method to improve cartilage degradation and prevent bone deterioration in patients with inflammatory TMJ diseases.

Mesenchymal stem cells (MSCs) have profound effects on immune modulation. Dental pulp stem cells (DPSCs) participate in systemic immune regulation and inflammation suppression (Yamaza et al. 2010). During immunomodulation by MSCs, signal transducer and activator of transcription 1 (STAT1) plays a critical role (Liu et al. 2015). Furthermore, STAT1 was demonstrated to participate in the regulation of MMPs under an inflammatory condition (Ala-aho et al. 2000; Cutler et al. 2017). However, whether the STAT1 pathway plays a vital role in MSC-based therapy for inflammatory TMJ diseases remains to be elucidated. We previously established 2 TMJ disorder models in rats via intra-articular injection of chemical agents. The models included a cartilage destruction model induced by monosodium iodoacetate (MIA; Wang et al. 2013) and a chronic inflammation model induced by complete Freund's adjuvant (CFA; Wang et al. 2012). Our previous study also showed that progressive TMJ arthritis could be established by the combined injection of MIA and CFA (Xue et al. 2018). This study aimed to evaluate the therapeutic effect of intra-articular injection of DPSCs on progressive TMJ arthritis in rats and to clarify the role of the STAT1 pathway in disease progression.

Materials and Methods

Animals

Seven-week-old female Sprague-Dawley rats (180 to 200 g) were purchased from Vital River Laboratory. To evaluate the therapeutic effects of DPSCs, the rats were randomly divided into 3 groups ($n = 5$): control, arthritis + normal saline (NS), and arthritis + DPSCs. The TMJs were harvested on days 14 and 28 after induction of arthritis.

To determine the possible target for DPSC treatment in the early stage of arthritis, the rats were randomly divided into the control and arthritis groups ($n = 3$). The TMJs were harvested on day 6 after induction of arthritis. The study was authorized by the Peking University Animal Ethics Committee (LA2014221). At room temperature, 5 rats were placed on a 12-h light/dark cycle in a cage with a specific pathogen–free environment.

Induction of TMJ Arthritis

On day 0, 0.5 mg of MIA (Sigma) dissolved in 25 μL of NS was mixed with 25 μL of CFA (Sigma). The mixture (50 μL) was injected intra-articularly to induce TMJ arthritis (Xue et al. 2018). The control group was injected with an equivalent amount of NS.

Isolation, Culture, and Injection of Human DPSCs

Protocols used to acquire human tissues were previously described (Xin et al. 2018) and conducted upon obtaining approval from the Ethical Guidelines of Peking University and informed consent from participants (PKUSSIRB-201311103). DPSCs were dissected from the extracted premolars of healthy donors and cultured with alpha modification of Eagle's medium (containing 15% fetal bovine serum) at 37 °C with 5% CO_2. DPSCs were identified according to a previously described method (Xin et al. 2018), and cells with 3 passages were used.

Prior to injection, DPSCs were digested with 0.25% trypsin (Hyclone), collected, and counted. On day 6 after CFA + MIA injection, 2×10^5 cells diluted in saline were injected per joint in the arthritis + DPSCs group. Rats in the control and arthritis + NS groups were injected with an equivalent amount of saline.

Measurement of Head Withdrawal Threshold

Head withdrawal threshold (HWT) was measured to evaluate the inflammatory hyperalgesia of TMJ according to our previously reported method (Kou et al. 2011; Wang et al. 2012). In brief, the rats stood on their hindpaws in the researcher's hand, covered by a working glove. During the test, the animals were kept unmoved without any strain from external force. The minimum threshold force applied on the TMJ that causes sudden head withdrawal was recorded by an electronic von Frey anesthesiometer (IITC Life Science). All measurements were performed by researchers who were blinded to the groups. Six HWT measurements were taken: at baseline (1 d prior to CFA + MIA/saline injections) and on days 1, 5, 7, 11, and 21.

Histology and Scoring of TMJ Arthritis

TMJs were simultaneously removed en bloc as described previously (Wang et al. 2012). The specimens were fixed in 10% formalin and decalcified in 10% EDTA. After being embedded in paraffin, the sections (5 μm) were stained with toluidine blue or hematoxylin and eosin. The degree of TMJ arthritis was scored by 3 experienced researchers who were blinded to the groups. Four sections, each from a different animal, were selected to evaluate the pathologic changes in TMJ. According to the modified Mankin scoring system and inflammation criteria from previous studies, the scoring system was divided into 2 parts—synovial inflammation and cartilage and bone deterioration (Appendix Table 1; Kapila et al. 1995; Kou et al. 2011; Xue et al. 2018). The scores given by the researchers for every sample were averaged. High scores indicate severe synovial inflammation and cartilage and bone deterioration.

Treatment of Rat Fibroblast-like Synoviocytes

The cultured rat fibroblast-like synoviocytes (FLSs) were seeded into 6-well plates for 2×10^5 cells/well. The culture medium was added with 10-ng/mL TNF-α and 10-ng/mL

IFN-γ (Peprotech) at 1, 24, and 72 h. For the coculture system, DPSCs were seeded at 10^5 cells per well into a Transwell upper chamber (pore size: 0.4 μm), and FLSs were seeded into the lower chamber. The STAT1 antagonist fludarabine (50 μM) was added 2 h prior to TNF-α and IFN-γ treatment to block the downstream function of the STAT1 pathway (Frank et al. 1999; Wei et al. 2019). Dimethyl sulfoxide was added into the culture medium as contrast for other groups.

The following methods are described in detail in the Appendix:

- Micro–computed tomography (micro-CT) examination
- Isolation, culture, and identification of rat FLSs
- Immunohistochemistry staining
- Immunofluorescence staining
- Western blot analysis
- Real-time polymerase chain reaction

Statistical Analysis

Statistical analysis was performed with SPSS 20.0 (IBM). The Kolmogorov-Smirnov test was used to judge whether the data conform to normal distribution. Based on the data distribution, differences were assessed by 1-way analysis of variance with Tukey's test for HWT measurement and the Holm-Sidak test for immunohistochemistry-positive cell counting, Western blot, and polymerase chain reaction analysis. The Kruskal-Wallis nonparametric test was used for micro-CT analysis and histological scoring, and P values <0.05 were considered statistically significant.

Results

DPSCs Relieved Hyperalgesia of Progressive TMJ Arthritis

Multiple HWT measurements were conducted before and after CFA + MIA or DPSC injection to estimate the pain level of rats during TMJ arthritis progression (Fig. 1A). The results of baseline (1 day prior to day 0) showed no significant differences among the 3 groups (Fig. 1B). After arthritis induction, the HWT values in the arthritis + NS and arthritis + DPSCs groups significantly decreased as compared with the control group on days 1 and 5 ($P < 0.01$). After the injection of DPSCs, the HWT value increased in the arthritis + DPSCs group and was significantly different from that in the arthritis + NS group on day 7 ($P < 0.05$). The HWT value in the arthritis + NS group remained at a low level until day 11. The changes in the HWT revealed that the local injection of DPSCs effectively relieved hyperalgesia of progressive TMJ arthritis

DPSCs Attenuated Bone Deterioration of Progressive TMJ Arthritis

Micro-CT examination was delivered, and the volume of interest was chosen on the anterior slope of the condyle to calculate the bone-related parameters (Fig. 1C). The results demonstrated the continuous and smooth surface of condyles and the even distribution of subchondral trabecular bone in the control group (Fig. 1D, E). Severe bone deterioration was detected 2 wk after inflammation induction, especially in the anterior slope of the condyle. Moreover, the subchondral bone surface was discontinuous with decreased trabecular bone and large regional bone defect after 4 wk (Fig. 1F, G). Local injection of DPSCs effectively attenuated the abnormal bone remodeling after inflammation induction. As compared with the arthritis + NS group, the arthritis + DPSCs group had more bone volume in the anterior slope, more trabecular bone, and milder subchondral bone defect ($n = 4$).

DPSCs Attenuated the Degenerative Changes in Progressive TMJ Arthritis

The pathohistologic examination result showed that the synovium in the control group was thin and had several layers of synovial lining cells (Fig. 2A). The thickening of the subsynovial connective tissue led to the massive infiltration of mononuclear cells with liquefactive necrosis in the arthritis + NS group in 2 wk and lasted for 4 wk. Apart from the differences in synovial inflammation, the arthritis + NS group had loss of peripheral chondrocytes and subchondral bone erosion and exhibited progressive destruction for 4 wk. Toluidine blue staining showed corresponding loss of the cartilage matrix in the anterior slope of the condyle in the arthritis + NS group. Injection of DPSCs partially reversed the pathologic changes in the synovial inflammation. The extent of cartilage destruction was limited in the arthritis + DPSCs group as compared with the arthritis + NS group. Moreover, the arthritis + DPSCs group showed slightly irregular arrangement of chondrocytes and continuous staining of the matrix. Abnormal bone remodeling in TMJ arthritis was regulated by DPSCs (Appendix Fig. 2). The scores of cartilage and bone degenerative changes significantly increased in the arthritis + NS group ($P < 0.05$) as compared with the control group (Fig. 2B). The scores of the arthritis + DPSCs group decreased significantly as compared with the arthritis + NS group but remained higher than those in the control group ($P < 0.05$).

DPSCs Suppressed Local Immune Response and Partially Downregulated the Expression of MMP3 and MMP13 in Progressive TMJ Arthritis

The level of $CD4^+$ T cells was detected as a marker to evaluate local immune response (Scotece et al. 2017). In the control group, a minute quantity of $CD4^+$ T cells was observed in the synovial tissue and subchondral bone marrow. After TMJ arthritis induction, the quantity of $CD4^+$ T cells was significantly elevated (Fig. 3A). DPSC injection partially suppressed the overexpression of $CD4^+$ T cells ($P < 0.05$). The results indicated the immunoregulatory effects of DPSCs. Moreover, IFN-γ and TNF-α exhibited high specific expression in the synovial lining cell in the arthritis + NS group (Fig. 3B). The arthritis + DPSCs group demonstrated a similar trend, wherein DPSCs partially downregulated the expression of IFN-γ and TNF-α. In

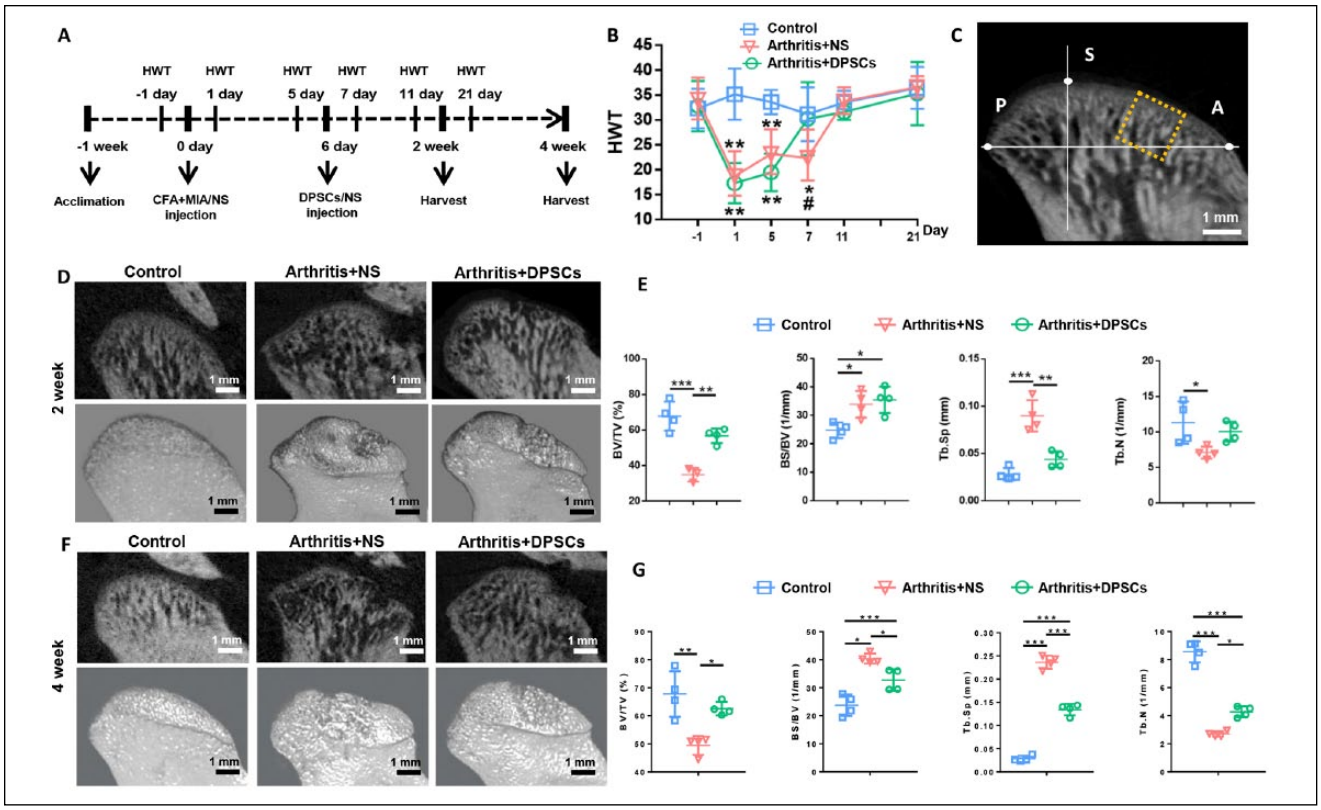

Figure 1. Dental pulp stem cell (DPSC) local injection effectively relieved the pain and bone destruction of progressive temporomandibular joint (TMJ) arthritis. (**A**) Timeline of induction, treatment, and behavioral assessment of rat TMJ arthritis. (**B**) The head withdrawal threshold (HWT) was significantly lower 1 d after complete Freund's adjuvant (CFA) + monosodium iodoacetate (MIA) injection as compared with the control group, and the arthritis + DPSCs group showed significant recovery to baseline 1 d after DPSC local injection as compared with the arthritis + normal saline (NS) group. *Versus control group. #Versus arthritis + NS group ($n = 5$). (**C**) Schematic diagram of volume of interest (VOI). A cuboid with 1.3 × 1.3 × 0.3–mm volume on the anterior inclined plane of the condyle was chosen to calculate bone-related parameters. The yellow box represents the cross section of VOI: A, most anterior position; P, most posterior position; S, most superficial position of condyle head. (**D**, **F**) Representative sagittal view and 3-dimensional reconstruction for 2-wk (D) and 4-wk (F) samples from temporomandibular condyles by micro–computed tomography. The arthritis + NS group showed bone defects on the anterior inclined plane, and subchondral bone remodeling was more severe for 4 wk than 2 wk. Bone deterioration of the arthritis + DPSCs group was better than that of the arthritis + NS group for 4 wk and 2 wk. (**E**, **G**) Statistical analysis of bone deterioration relative parameters for 2 wk (E) and 4 wk (G). There were significant differences between the control and arthritis + NS groups, while the arthritis + DPSCs group partially reversed the bone deterioration. Data of the HWT measurement are presented by mean ± SD; data of micro–computed tomography analysis are presented by median with interquartile range (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; $n = 4$).

addition, the expression of MMP3 and MMP13 were detected to evaluate the cartilage destruction under an inflammatory condition (Fig. 3C, D). In the control group, MMP3 and MMP13 were expressed in the synovium and condyle, representing active bone and cartilage remodeling under physiologic conditions. The expression of MMP3 and MMP13 was significantly upregulated in the arthritis + NS group. The upregulation of MMP3 and MMP13 was partly blocked by DPSC injection ($P < 0.05$).

DPSC Coculture Blocked MMP3 and MMP13 Expression in FLSs Induced by Inflammatory Cytokines

The coculture system was used to elucidate the influences of DPSCs on FLSs under an inflammatory condition. According to the results of immunohistochemistry staining of the in vivo study, IFN-γ and TNF-α were used to induce the inflammatory reaction of FLSs. Immunofluorescent staining showed high expression of MMP3 and MMP13 in the cytoplasm of FLSs after continuous stimulation of inflammatory cytokines (Fig. 4A). The high expression was reversed by coculture with DPSCs for 72 h. Gene expression analysis demonstrated the regulatory effects of DPSCs on *Mmp3* and *Mmp13* (Fig. 4B). The gene expression of *Timp1*, an MMP inhibitor, increased in the DPSC coculture system. This trend further explained the regulatory effects of DPSCs on matrix metabolism. The Western blot results confirmed the changes in MMP3 and MMP13 expression under inflammatory condition (Fig. 4C). DPSCs reversed the reactive upregulation of MMP3 and MMP13.

DPSCs Regulated MMP3 and MMP13 Expression in FLSs through the STAT1 Pathway

To explore the possible mechanism of progressive TMJ arthritis and find the possible target for DPSCs, the activation of the

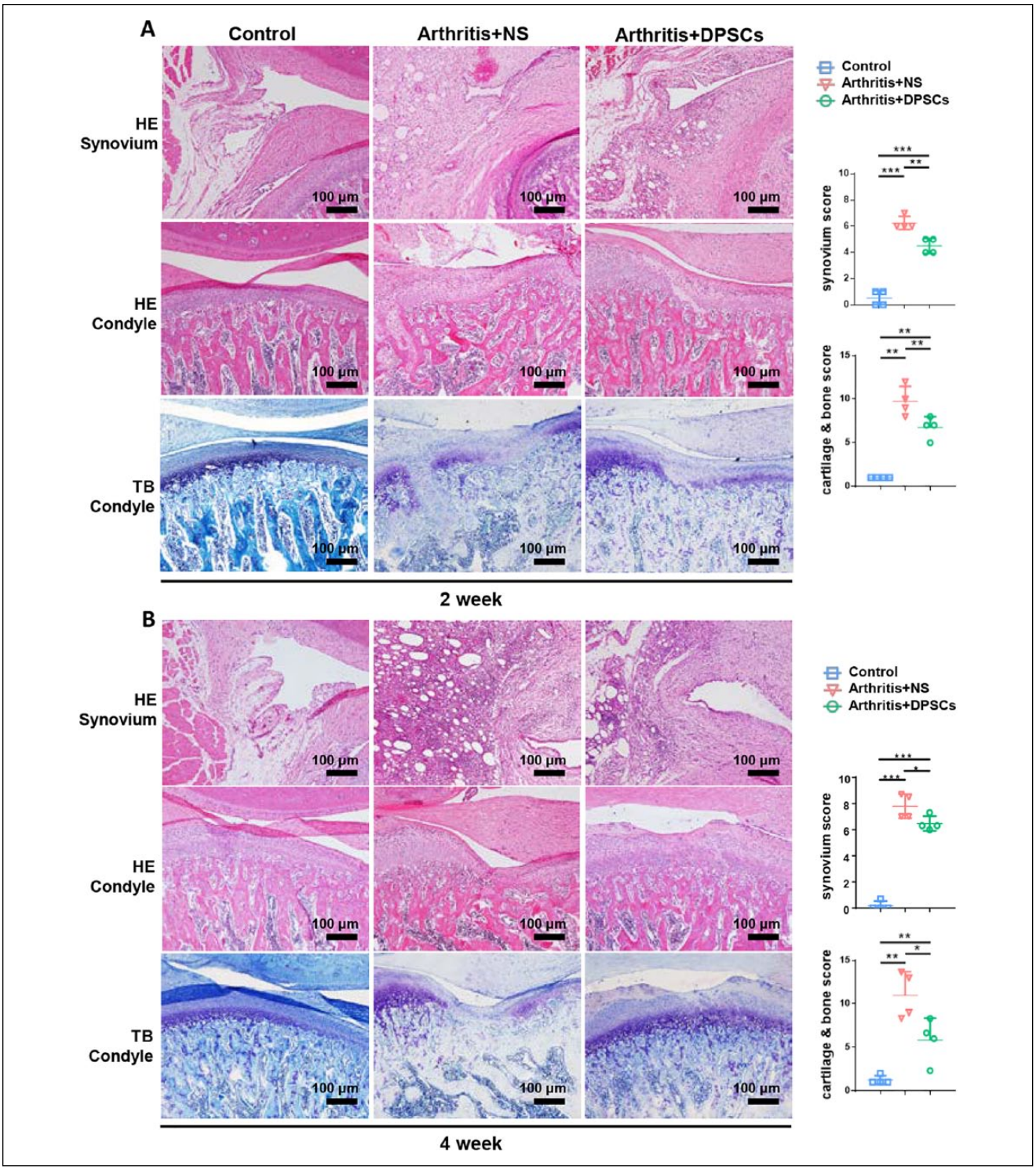

Figure 2. Dental pulp stem cell (DPSC) local injection improved pathologic structural damages of temporomandibular joint (TMJ) arthritis. (**A**) Representative images of hematoxylin-eosin (HE) and toluidine blue (TB) staining of TMJ. When compared with the control group, the arthritis + normal saline (NS) group showed thickening of subsynovial connective tissue, accompanied with cartilage disconnection and subchondral bone deterioration. The synovial inflammation existed continuously from 2 wk until 4 wk. Meanwhile, cartilage and bone defects were progressive with the extension of time. For 2 wk and 4 wk, the arthritis + DPSCs group showed minor inflammatory cells infiltration, with slight bone destruction. (**B**) Pathologic scores of synovium and cartilage and bone score of TMJ. The scores of the arthritis + NS group were significantly higher than those of control group, and the pathologic damage of the arthritis + DPSCs group was much milder than that of the arthritis + NS group. Data are presented by median with interquartile range (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; $n = 4$).

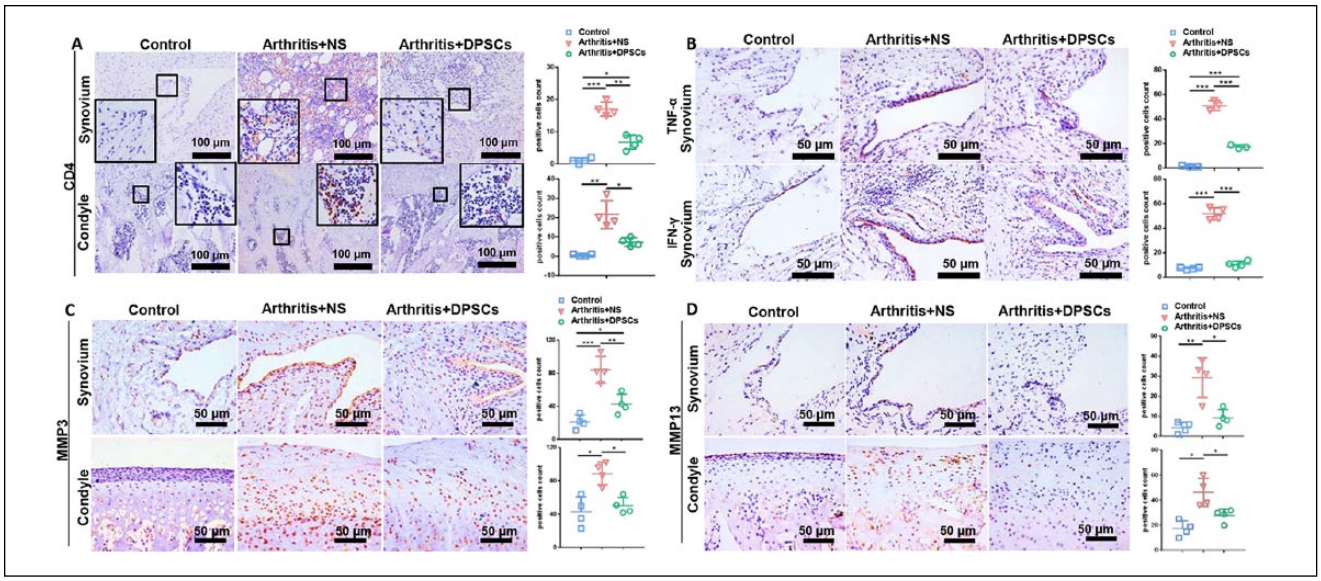

Figure 3. Dental pulp stem cells (DPSCs) attenuated temporomandibular joint (TMJ) arthritis by modulating local immune inflammatory response and cartilage matrix metabolism. (**A**) Representative immunohistochemical images of synovium and condyle. The quantity of $CD4^+$ T cells was significantly upregulated in the arthritis + NS group and decreased in the arthritis + DPSCs group. (**B**) The expression of TNF-α and IFN-γ in synoviocytes was upregulated in the arthritis + normal saline (NS) group and decreased in the arthritis + DPSCs group. (**C**, **D**) Representative immunohistochemical images of condylar cartilage and synovium. The expression of MMP3 and MMP13 was upregulated in the arthritis + NS group and showed no significant difference between the arthritis + DPSCs group and the control group. Data are presented by mean ± SD (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; $n = 4$).

Janus kinase 2 (JAK2)–STAT1 pathway in synovium from the control and arthritis groups was detected on day 6 after CFA + MIA injection (Fig. 5A). The expression of JAK2, phosphorylated STAT1 (p-STAT1), and STAT1 was elevated in the inflammatory synovium, which indicated that the activation of the JAK-STAT pathway may be a target for DPSC-based therapy. The high expression of the p-STAT1 was also detected in the synovium 4 wk after inflammation was induced (Fig. 5B). DPSC injection blocked the activation of STAT1 in vivo. To clarify the role of the STAT1 pathway in synoviocytes, the STAT1 expression was detected under the inflammatory condition in vitro (Fig. 5C, D). After 24 and 72 h of stimulation with inflammatory cytokines, the expression of total STAT1 in FLSs significantly increased in the inflammatory environment, and DPSCs reversed the upregulation of total STAT1. DPSCs inhibited the activation of STAT1 by downregulating the phosphorylation of the Tyr 701 site (Fig. 5E, F). Fludarabine was used to block the STAT1 activation and clarify the relationship among STAT1, MMP3, and MMP13 (Fig. 5G, H). The results demonstrated that fludarabine blocked STAT1 expression after inflammatory cytokine stimulation for 24 h. The expression of MMP3 and MMP13 showed the same trend as STAT1.

Discussion

In this study, local injection of DPSCs attenuated CFA + MIA–induced progressive TMJ arthritis in rats. DPSCs remarkably ameliorated the clinical pain and degenerative changes in progressive TMJ arthritis. In terms of mechanism, the activation of the STAT1 pathway participated in the regulation of MMPs during the progression of TMJ arthritis. DPSCs effectively inhibited the activation of STAT1, resulting in downregulation of MMP3 and MMP13 (Appendix Fig. 3). This study proposes that DPSCs attenuate the pathologic changes of experimental progressive TMJ arthritis and provide a new prospective treatment for patients with inflammatory TMJ diseases.

Immune inflammatory response plays a crucial role in the pathogenesis of TMJ arthritis as compared with other joints, such as the knee joint (Vos et al. 2014). Activation of immune cells and elevated expression of multiple inflammatory factors were intensively related to the development of inflammatory OA (Penatti et al. 2017; Monasterio et al. 2018). In this study, the inhibition of $CD4^+$ T cells by DPSCs was possibly related to the MSC-induced T-cell apoptosis (Yang et al. 2018). The suppression of immune cells could ameliorate synovial inflammation and further influence the function of TMJ resident cells.

Increasing evidence suggests the critical role of synovitis and the subsequent proinflammatory cytokines in the pathogenesis of cartilage degradation (Mathiessen and Conaghan 2017). Severe synovial inflammation is mainly presented in autoimmune arthritis, such as rheumatoid arthritis, and induces resultant bone and cartilage deterioration (Huh et al. 2015). By contrast, opinions on the occurrence of synovitis in patients with OA are controversial. For progressive or late-stage OA, synovial inflammation was typical and related to the severity of cartilage destruction (de Lange-Brokaar et al. 2012). However, in early OA, conflicting results are noted to detect synovitis by macroscopic or radiographic tests probably due to the low degree of inflammation. In the present study, intra-articular injection of CFA + MIA can simulate synovial inflammation and cartilage destruction in TMJ. As such, the 2 types of

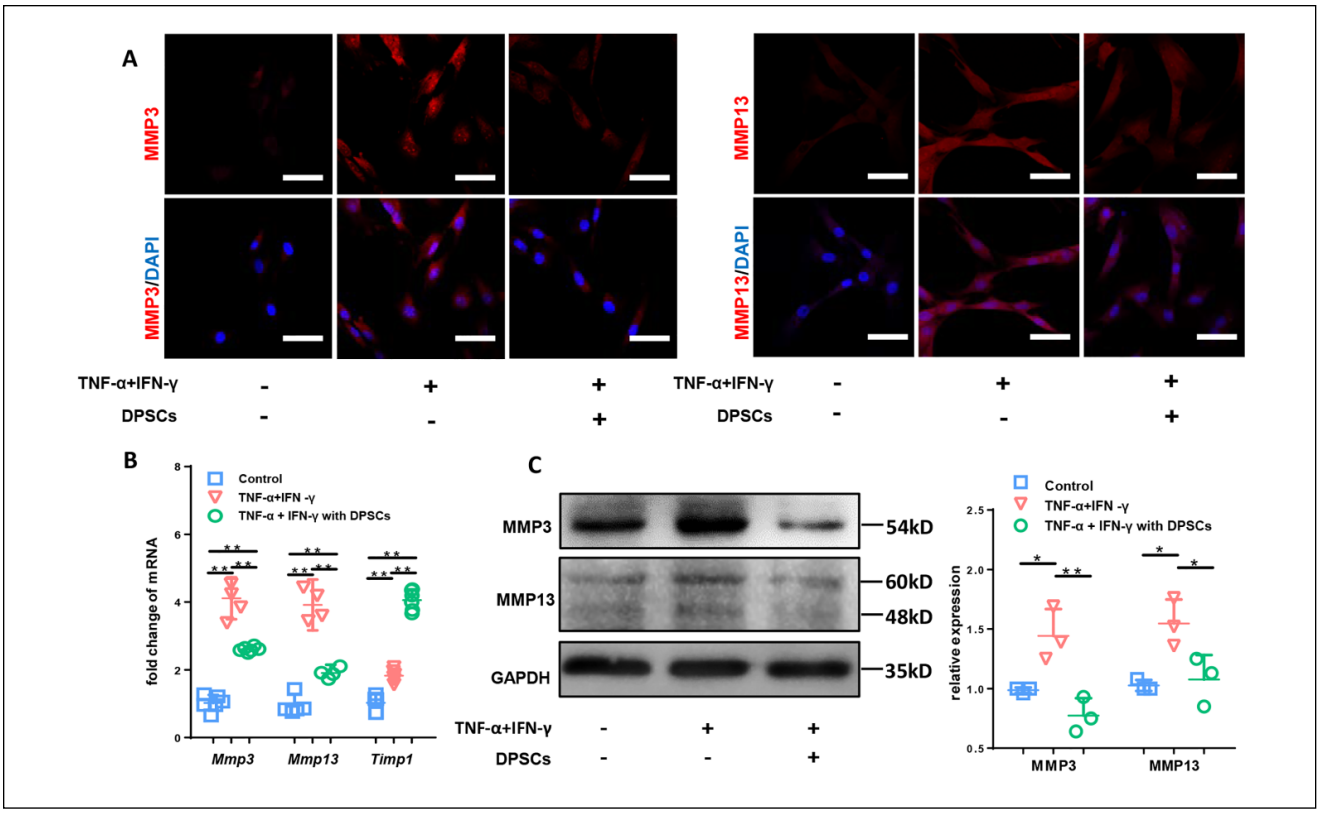

Figure 4. Dental pulp stem cells (DPSCs) downregulated the expression of MMP3 and MMP13 in fibroblast-like synoviocytes under the inflammatory condition. (**A**) Immunofluorescence of fibroblast-like synoviocytes showed that the higher expression of MMP3 and MMP13 under inflammatory condition was partial reversed by DPSC coculture for 72 h (bar: 50 μm). (**B**) Expression of MMP3, MMP13, and TIMP1 mRNA assessed by polymerase chain reaction analysis was increased under the inflammatory condition, and DPSC coculture for 72 h decreased the expression of MMP3 and MMP13 and promoted the expression of TIMP1 ($n = 6$). (**C**) Western blot from 3 independent tests showed that the expression of MMP3 (54 kD) and MMP13 (precursor: 60 kD, active domains: 48 kD) was significantly upregulated under the inflammatory condition and the tendency was reversed by DPSCs. Data are presented by mean ± SD (*$P < 0.05$, **$P < 0.01$).

chemical agents were selected to build the in vivo model to mimic the progressive synovitis, severe cartilage, and bone destruction in TMJ arthritis.

Treatment for improving bone and cartilage destruction in TMJ arthritis with progressive synovial inflammation is difficult. As previous studies reported, systemic application of MSCs was demonstrated to be effective in autoimmune arthritis (Park et al. 2018). Therefore, we conducted local and systemic injections of DPSCs in rats with progressive TMJ arthritis. However, systemic injection showed no significant improvement in TMJ arthritis (Appendix Fig. 4). Thus, localized arthritis was sensitive to the local microenvironment, and topical administration was better than systemic administration for treatment.

Locally injected bone marrow mesenchymal stem cells were reported to differentiate and participate in cartilage repair in TMJ (Zhang et al. 2017; Zhang et al. 2019). As compared with bone marrow mesenchymal stem cells, DPSCs are easily acquired and show strong potential of immunomodulatory effects (Zhao et al. 2012). The severity of TMJ arthritis in the present study was more serious than that reported in previous studies. With its good therapeutic effects, local injection of DPSCs could be a new method for improving drug injection therapy for patients with severe TMJ degenerative changes.

The expression of MMPs was at a certain level in condylar cartilage to maintain the balance of matrix synthesis and degradation (Ye et al. 1996). Thus, suppression of abnormally highly expressed MMP3 and MMP13 contributed to the improvement of cartilage degradation in inflammatory TMJ diseases. In addition, the gene expression of *Mmp1* and *Mmp9* was detected in FLSs (Appendix Fig. 5A). However, the regulatory effects of DPSCs on *Mmp1* and *Mmp9* were not substantial as compared with the results of *Mmp3* and *Mmp13*. The limited downregulation of DPSCs on other MMPs suggests that the function of the MMP family was complicated. Thus, further studies should be conducted to elucidate MSC-based therapy.

The suppression of the JAK-STAT1 pathway effectively downregulated the activation of immune cells and provided a target for MSC-based therapy (Hertenstein et al. 2011; Dong et al. 2018). Our results suggest that STAT1 plays an important role in regulating the local microenvironment and provides a possible mechanism of the therapeutic effects of DPSCs. Moreover, STAT1 participates in the regulation of MMP13 and

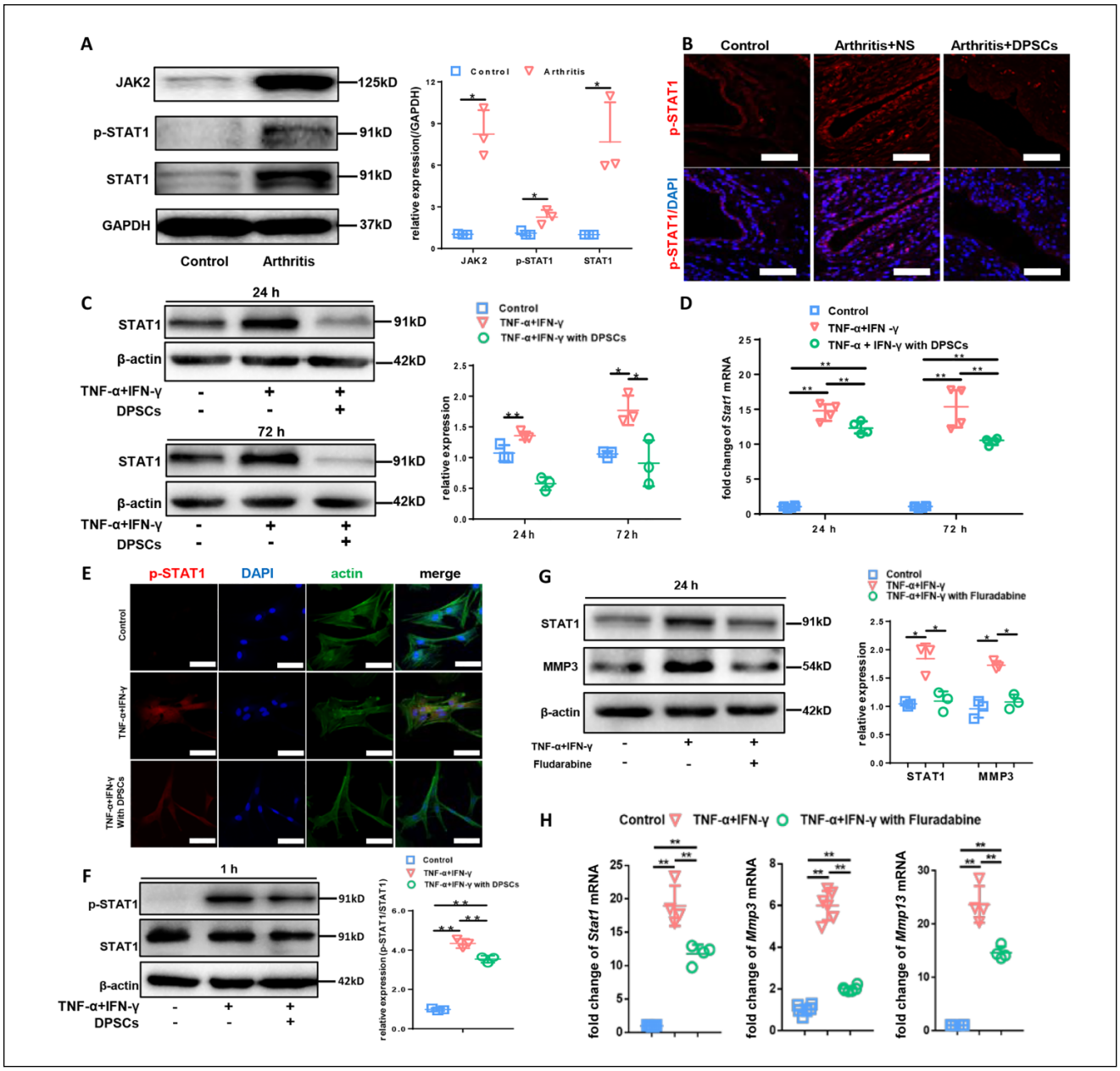

Figure 5. Dental pulp stem cells (DPSCs) regulated the expression of MMP3 and MMP13 by suppressing the expression and activation of STAT1 under the inflammatory condition. (**A**) Western blot results from temporomandibular joint (TMJ) synovial tissues 6 d after arthritis was induced. The expression of JAK2, p-STAT1, and STAT1 in the arthritis group showed significant upregulation as compared with the control group ($n = 3$). (**B**) Representative images of synovium 4 wk after induced TMJ arthritis. The expression of p-STAT1 was significantly upregulated in the arthritis + normal saline (NS) group and decreased in the arthritis + DPSCs group (bar: 20 μm). The expression of STAT1 was increased after being treated with inflammatory factors and also partially reversed by DPSC coculture for 72 h as detected by Western blot (**C**) from 3 independent tests and polymerase chain reaction analysis (**D**) ($n = 4$). Immunofluorescence staining (**E**) and Western blot (**F**) showed that phosphorylation and nuclear translocation of STAT1 were increased after being treated with inflammatory factors for 1 h and reversed by DPSC coculture (bar: 50 μm). Western blot (**G**) from 3 independent tests and polymerase chain reaction analysis (**H**) ($n = 4$-6) showed that the upregulation of *Stat1*, *Mmp3*, and *Mmp13* was partly blocked by STAT1 inhibitor fludarabine treated 2 h prior to inflammatory stimulation. Data are presented by mean ± SD (*$P < 0.05$, **$P < 0.01$).

type II collagen of chondrocytes (Huang et al. 2016). In the present study, blocking STAT1 inhibited the expression of *Mmp3* and *Mmp13* but was not effective on *Mmp9* (Appendix Fig. 5B). Thus, the expression of MMPs was not regulated by only the STAT1 pathway.

Although this study assessed the therapeutic effects of DPSCs on progressive TMJ arthritis, substantial work needs to be conducted to elucidate the mechanisms of regulation or even the crosstalk between DPSCs and synoviocytes. The secreted exosomes and microRNA of DPSCs inhibit inflammation and

induce tissue regeneration (Ishikawa et al. 2016; Yan et al. 2016; Chen et al. 2018) and could be the possible mediators for the effects of DPSCs on synoviocytes. However, despite the immune regulatory effects of MSCs, further studies are needed to estimate whether exogenous DPSCs could induce immune rejection and differentiate in the local environment.

In conclusion, local injection of DPSCs exhibited therapeutic effects on rats with TMJ arthritis by regulating MMP3 and MMP13 expression through the STAT1 pathway.

Author Contributions

S.J. Cui, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; T. Zhang, Y. Fu, contributed to data analysis and interpretation, critically revised the manuscript; Y. Liu, contributed to design and data interpretation, critically revised the manuscript; Y.H. Gan, Y.H. Zhou, contributed to conception, data analysis, and interpretation, critically revised the manuscript; R.L. Yang, X.D. Wang, contributed to conception, design, data acquisition, analysis, and interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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