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Original Article

Conditioned Medium from Stem Cells of Human Exfoliated Deciduous Teeth Partially Alters the Expression of Inflammation-associated Molecules of Mouse Condylar Chondrocytes via Secreted Frizzled-related Protein 1

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Abstract: Intravenous administration of conditioned medium from stem cells of human exfoliated deciduous teeth (SHED-CM) effectively restores mechanically injured osteochondral tissues in mouse temporomandibular joint osteoarthritis. However, the underlying therapeutic mechanisms remain elusive. Here, we investigated the direct therapeutic effects of SHED-CM on inflamed primary condylar chondrocytes *in vitro*. Immunofluorescence staining revealed that interleukin-1β-stimulated chondrocytes showed increased expression of the catabolic marker inducible nitric oxide synthase (iNOS) and reduced expression of the anabolic marker aggrecan (ACAN). We found that SHED-CM treatment, but not conditioned medium from bone marrow mesenchymal stem cells (BMSC-CM), effectively suppressed iNOS expression and elevated ACAN levels, indicating that SHED-CM converted the catabolic phenotype of inflamed chondrocytes to an anabolic phenotype. Liquid chromatography with tandem mass spectrometry analysis of SHED-CM and BMSC-CM identified eight proteins enriched in SHED-CM that are related to anti-inflammatory and/or chondrogenic processes. Of these proteins, the Wnt signal inhibitor secreted frizzled-related protein 1 (SFRP1) was the most abundantly enriched in SHED-CM. We found that treatment with the selective SFRP1 inhibitor WAY-316606 abolished the anti-catabolic and pro-anabolic effects of SHED-CM. Collectively, our study suggests that SHED-CM directly suppresses catabolism and promotes anabolic responses of inflamed primary condylar chondrocytes, which partially rely on SFRP1 function in SHED-CM. The direct action of SHED-CM may be useful to treat inflammatory cartilage diseases.

1. Introduction

Temporomandibular joint osteoarthritis (TMJOA) is the most common disease of the TMJ and is characterized by chondrocyte inflammation and death, extracellular matrix (ECM) degradation, and impaired subchondral bone remodeling[1]. Although the etiology of TMJOA remains unclear, excessive mechanical stress-induced cartilage lesions are considered a key factor in the initiation and progression of TMJOA[2,3]. Chondrocytes maintain a dynamic equilibrium between ECM synthesis and degradation. When enduring

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mechanical overload, homeostasis is disrupted by uncoupled catabolic and anabolic activities. Compressed chondrocytes release proinflammatory cytokines and matrix-degrading enzymes, such as inducible nitric oxide synthase (iNOS), interleukin-1β (IL-1β), and matrix metalloproteinase-13 (MMP13)[4, 5]. With the progression of TMJOA, chondrocyte death and cartilage matrix degradation further promote synovial inflammation and subchondral bone destruction, leading to irreversible joint tissue injuries[1, 6]. To date, effective treatments to repair tissue injuries in TMJOA are unavailable.

Recently, stem cell-based therapies have gained considerable attention in tissue engineering and regenerative medicine. Intra-articular transplantation of mesenchymal stem cells (MSCs) derived from the bone marrow (BMSCs), adipose tissue, or dental pulp effectively restored cartilage degeneration in preclinical studies of a rodent TMJOA model[7-9]. Notably, recent studies have demonstrated that the therapeutic effects of MSCs are largely attributed to the trophic and immunomodulatory activities of their paracrine secretions, and not to the restoration of lost cells by transplanted MSCs[10, 11]. It has been reported that local or systemic administration of the MSC secretome markedly alleviates cartilage injuries in knee OA and TMJOA[12, 13].

Stem cells from human exfoliated deciduous teeth (SHEDs) have gained much attention in recent years because of their strong paracrine therapeutic activities in various intractable disease models[14]. We have previously reported that conditioned medium from stem cells of human exfoliated deciduous teeth (SHED-CM) treatment ameliorates spinal cord injury, rheumatoid arthritis, and neuropathic pain[15-17]. Mechanical analysis revealed that SHED-CM restores injury by accelerating endogenous tissue-repairing activities, such as anti-inflammatory activity, angiogenesis, cellular protection, and induction of anti-inflammatory M2 macrophages. Recently, we also reported that intravenous administration of SHED-CM effectively restores mechanically injured cartilage in mouse TMJOA[18]. However, the underlying therapeutic mechanisms of SHED-CM in TMJOA remain unclear.

In this study, we examined the direct effects of SHED-CM on the catabolic and anabolic responses of inflamed primary condylar chondrocytes induced by IL-1β treatment and identified the therapeutic factors of SHED-CM using liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis. Herein, our data demonstrated that SHED-CM directly converted the catabolic phenotype of inflamed chondrocytes to an anabolic phenotype partially relying on secreted frizzled-related protein 1 (SFRP1) in SHED-CM.

2. Materials and methods

2.1. Preparation of CM

SHED-CM and BMSC-CM were prepared based on a previous study[19]. Briefly, exfoliated deciduous teeth were collected from six- to twelve-year-old individuals at Nagoya University and Tokushima University Hospital. The intentions of the donors were confirmed, and all parents or guardians provided written informed consent. All procedures were approved by the Institutional Ethical Committee of Nagoya University and Tokushima University Hospital (permit no. H-73 and no.3268 for Nagoya and Tokushima University, respectively). All experiments were conducted in accordance with the principles of the Declaration of Helsinki.

Dental pulp was collected and digested for 1 h at 37℃ in a solution containing collagenase type I (3 mg/mL) (FUJIFILM WAKO, Tokyo, Japan) and dispase (4 mg/mL) (FUJIFILM WAKO). Isolated cells were plated on culture dishes in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich, MO, USA), supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich), and incubated (Forma Direct Heat CO_2 Incubator, Thermo Fisher Scientific, MA, USA) at 37℃ in 5% CO_2. BMSCs at passage 5 were purchased from Lonza (PT-2501, Lonza, NC, USA).

At passages 5-9, SHEDs or BMSCs at 70-80% confluence were washed with phosphate-buffered saline (PBS) and cultured in serum-free DMEM. After incubation for 48 h, the medium was collected and centrifuged for 10 min at 2000 × *g* at 4℃. The supernatants were used as SHED-CM and BMSC-CM. The protein concentration in each CM was adjusted to 3 μg/mL using serum-free DMEM.

2.2. Isolation of primary condylar chondrocytes

Animal experiments were approved by the Animal Research Committees of Tokushima University (Permit No: T30-119) and were performed in accordance with the guidelines for animal experimentation of Tokushima University. All animal experiments were performed in accordance with ARRIVE guidelines. Primary chondrocytes of the mandibular condyle cartilage were isolated from five- to six-day-old newborn Institute of Cancer Research mice according to an established protocol[20]. Briefly, the cartilage of the TMJ was carefully dissected on a sterile bench and digested with a 3 mg/mL collagenase D solution (Roche Diagnostics GmbH, Mannheim, Germany) at 37℃ for 45 min. Thereafter, cartilage pieces were cultured with a 0.5 mg/mL collagenase D solution at 37℃ in a humidified 5% CO_2 atmosphere for 16 h. The next day, isolated chondrocytes were collected and replated with 10% FBS/DMEM. The culture medium was replaced every 2 d, and passage 2-3 were used in subsequent experiments.

2.3. IL-1β stimulation and CM treatment

Primary chondrocytes were seeded in 96-well plates. At approximately 70% confluence, chondrocytes were washed with PBS and serum-free DMEM and starved for 24 h. Thereafter, chondrocytes were divided into one sham control group and three experimental groups. In the experimental groups, chondrocytes were incubated with DMEM, BMSC-CM, or SHED-CM, supplemented with 10 ng/mL IL-1β (R&D, Bio-techne, MN, USA), at 37℃ in 5% CO_2 for 24 h.

2.4. Immunofluorescence staining

After 24 h of IL-1β stimulation, chondrocytes were fixed with 4% paraformaldehyde (PFA, NACALAI TESQUE, Kyoto, Japan) and permeabilized with 0.1% Triton-X-100 (FUJIFILM WAKO), followed by 30 min of blocking with 5% bovine serum albumin (FUJIFILM WAKO) in PBS. Primary antibodies against iNOS (1:500, ab3523, Abcam, Cambridge, UK), and Aggrecan (ACAN) (1:500, ab36861, Abcam) were incubated with chondrocytes for 1 h at 24-25℃. Next, chondrocytes were stained with fluorescence-conjugated secondary antibody (1:500, A11034, Thermo Fisher Scientific, MA, USA) and 4',6-diamidino-2-phenylindole (DAPI, 1:500, Sigma-Aldrich) at 24-25 ℃ for 1 h. Cell images were captured with a fluorescence microscope (BZ-X800, Keyence, Osaka, Japan) and the positive cells were counted.

2.5. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis

Secretome analysis of SHED-CM and BMSC-CM was performed using a previously reported protocol[21]. Briefly, each CM was concentrated using Amicon Ultra 3 K filters (MilliporeSigma, MA, USA), followed by methanol/chloroform precipitation to purify the proteins. Thereafter, protein pellets were resolved with MS buffer (8 M urea and 50 mM Tris-HCl, pH 8.0), reduced with 5 mM dithiothreitol (FUJIFILM WAKO) for 30 min at 24-25℃, and alkylated with 27.5 mM iodoacetamide (FUJIFILM WAKO) for 30 min in the dark at 24-25℃. Harvested proteins were diluted eight times with 50 mM Tris-HCl pH 8.0 and digested using Lys-C (FUJIFILM WAKO) and trypsin (Promega, WI, USA) with 1/100 of the sample protein volume for 8 h at 37℃. The next day, peptides were purified with GL-Tips SDB (GL Sciences, Tokyo, Japan), and their concentrations were examined using a Pierce™ quantitative colorimetric peptide assay kit (Thermo Fisher Scientific). Each sample (300 ng of peptides) was injected into an EASY-nLC™ connected to a Orbitrap fusion™ mass spectrometry system (Thermo Fisher Scientific). The scan range was set to m/z 350-1500. Protein identification, label-free quantification, production of hierarchical clustering, dendrograms, and heatmaps were conducted using Proteome Discoverer 2.4™ (Thermo Fisher Scientific).

2.6. Secreted frizzled-related protein 1 (SFRP1) blocking assay

To investigate the potential functions of SFRP1 in SHED-CM, the selective SFRP1 inhibitor WAY-316606 (Selleck Biotech, TX, USA) was used to block SFRP1 function. At approximately 70% confluence, condylar chondrocytes were starved for 24 h in serum-free DMEM and subsequently stimulated with IL-1β in DMEM or SHED-CM with/without 10 μM WAY-316606 for 24 h at 37℃. The cells were fixed with 4% PFA and subjected to immunofluorescence staining.

2.7. Statistical analysis

An unpaired two-tailed Student's *t*-test was used to compare the differences between two groups. A one-way analysis of variance (ANOVA) with Tukey's multiple comparisons test was used to compare the differences among three or more independent groups (GraphPad Prism version 9.4.0). $p < 0.05$ was considered statistically significant.

3. Results

3.1. SHED-CM treatment suppressed iNOS expression while elevating ACAN expression in IL-1β-stimulated primary condylar chondrocytes

We examined the effects of SHED-CM on inflamed chondrocytes. Mouse primary chondrocytes were stimulated with IL-1β in DMEM, BMSC-CM, or SHED-CM. Immunofluorescence staining showed that primary chondrocytes expressed ACAN. After IL-1β stimulation, they enhanced the expression of proinflammatory iNOS and suppressed that of ACAN. Notably, SHED-CM, and not BMSC-CM, significantly restored ACAN expression and suppressed iNOS expression (Fig. 1A and B; iNOS, DMEM vs. SHED-CM, $p < 0.001$; BMSC-CM vs. SHED-CM, $p < 0.01$; ACAN, DMEM vs. SHED-CM, $p < 0.001$; BMSC-CM vs. SHED-CM, $p < 0.001$). These results demonstrated that SHED-CM directly converted the catabolic phenotype of inflamed chondrocytes to an anabolic phenotype.

3.2. Secretome analysis of SHED-CM vs. BMSC-CM

We examined the protein expression profile of SHED-CM and BMSC-CM using LC-MS/MS analysis. A total of 1750 proteins were identified, of which 28 membrane-located or extracellular proteins in SHED-CM were expressed more than 10 times compared to those in BMSC-CM (Fig. 2A and B). Notably, among the 28 secreted proteins, eight were anti-inflammatory and/or pro-chondrogenic proteins-SFRP1, platelet-derived growth factor D (PDGFD), transforming

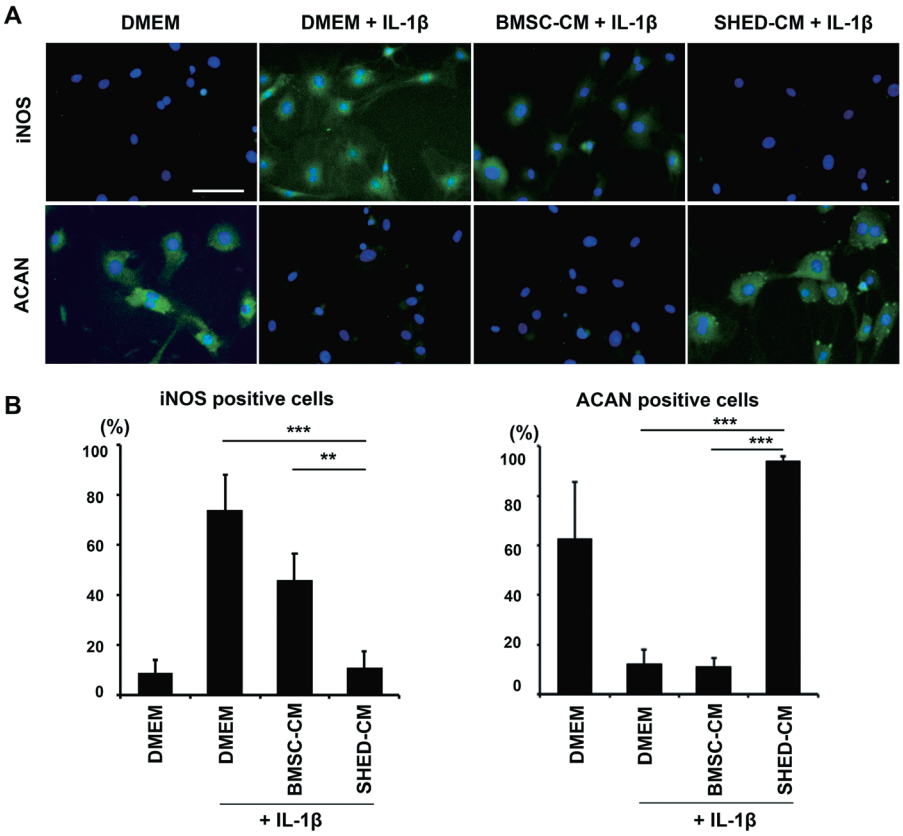

Fig. 1 SHED-CM treatment suppresses iNOS while enhancing ACAN expression in IL-1β-stimulated primary mandibular chondrocytes.
(A) Immunofluorescence images of iNOS and ACAN.
(B) Percentage of iNOS-, and ACAN-positive chondrocytes in DAPI-positive. Similar results were obtained from two independent experiments.
Data represent mean ± SD. $^{**}p < 0.01$, $^{***}p < 0.001$. Scale bar = 100 μm.

growth factor beta-2 (TGFB2), secreted frizzled-related protein 3 (FRZB), matrix-remodeling-associated protein 5 (MXRA5), alpha-2-macroglobulin (A2M), semaphorin-3A (SEMA3A), and midkine (MDK). Notably, SFRP1 expression was remarkably higher in SHED-CM than that in BMSC-CM (Fig. 2B and C).

3.3. Blocking of SFRP1 reduced the anti-inflammatory and pro-anabolic effects of SHED-CM

As the Wnt antagonist SFRP1 was the most abundant protein enriched in SHED-CM and has been reported to regulate the catabolic/anabolic phenotype of chondrocytes[22], we hypothesized that SFRP1 may participate in the SHED-CM-mediated regulation of chondrocyte phenotype. To block the SFRP1 function in SHED-CM, we used a selective SFRP1 inhibitor, WAY-316606. Immunofluorescence staining demonstrated that WAY-316606 treatment alone did not affect the catabolic/anabolic phenotype of chondrocytes, however it significantly increased iNOS expression and decreased ACAN expression in SHED-CM-treated inflamed chondrocytes (Fig. 3A and B, iNOS, SHED-CM vs. SHED-CM/WAY, $p < 0.001$; ACAN, SHED-CM vs. SHED-CM/WAY, $p < 0.0001$). These results demonstrated that SFRP1 in SHED-CM plays a major role in the conversion of catabolic to anabolic phenotypes of inflamed chondrocytes (Fig. 4).

Discussion

In this study, we examined the direct effects of SHED-CM on inflamed condylar chondrocytes *in vitro*. Our data showed that SHED-CM directly converted the catabolic phenotype of inflamed chondrocytes to an anabolic phenotype. This observation supports the notion that SHED-CM injected into the tail vein can reach and directly repair the injured TMJ. However, importantly, we observed that the therapeutic effects of SHED-CM on several disease models were attributed to the anti-inflammatory M2 macrophages induced

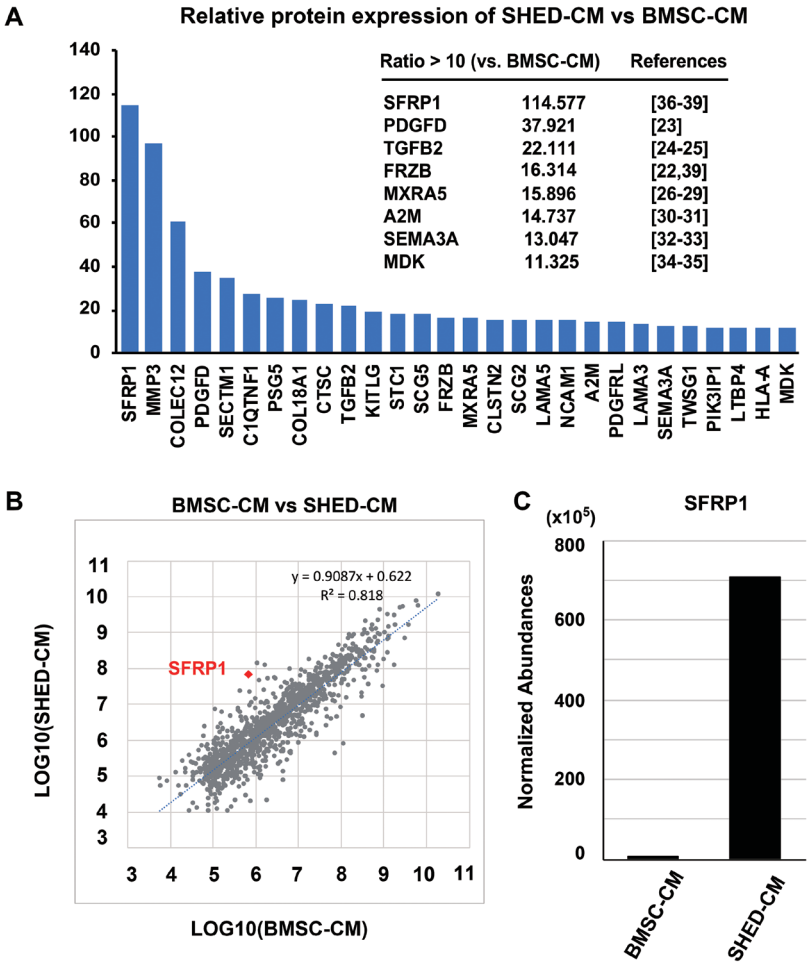

Fig. 2 List of extracellular proteins identified by secretome analysis using mass spectrometry. Identified proteins were quantified using normalized abundance.
(A) List of proteins that were expressed more than10 times in SHED-CM relative to BMSC-CM. Eight proteins in SHED-CM were identified to participate in anti-inflammation and/or pro-chondrogenesis activities.
(B) Scatter plot based on quantification of all proteins in SHED-CM and BMSC-CM. The linear function and correlation coefficient R-squared values indicate the relationship between the quantitative values of two types of CM. The red diamond indicates SFRP1.
(C) Normalized abundance of SFRP1 in SHED-CM and BMSC-CM.

by SHED-CM. For example, administration of SHED-CM into the tail vein ameliorates neuropathic pain in a mouse sciatic nerve ligation model, which is accompanied by the induction of M2 macrophages at the site of nerve ligation and the dorsal root ganglion[17]. Notably, specific depletion of M2 by mannosylated-clodrosome markedly reduces the antinociceptive effect of SHED-CM. Furthermore, intravenous administration of conditioned medium from M2-induced SHED-CM ameliorates neuropathic pain[17]. The study demonstrates the remarkable therapeutic activity of M2-induced by SHED-CM. In future, to understand the mechanical basis of the SHED-CM-mediated amelioration of TMJOA, it is worth investigating the possibility that SHED-CM acts on cells other than damaged chondrocytes and indirectly repairs cartilage damage in TMJOA.

In this study, we examined the protein profile of each CM using LC-MS/MS analysis and detected 28 membrane-located or secreted proteins that were expressed more than 10 times in SHED-CM compared to BMSC-CM. Based on previous studies, eight proteins related to anti-inflammation

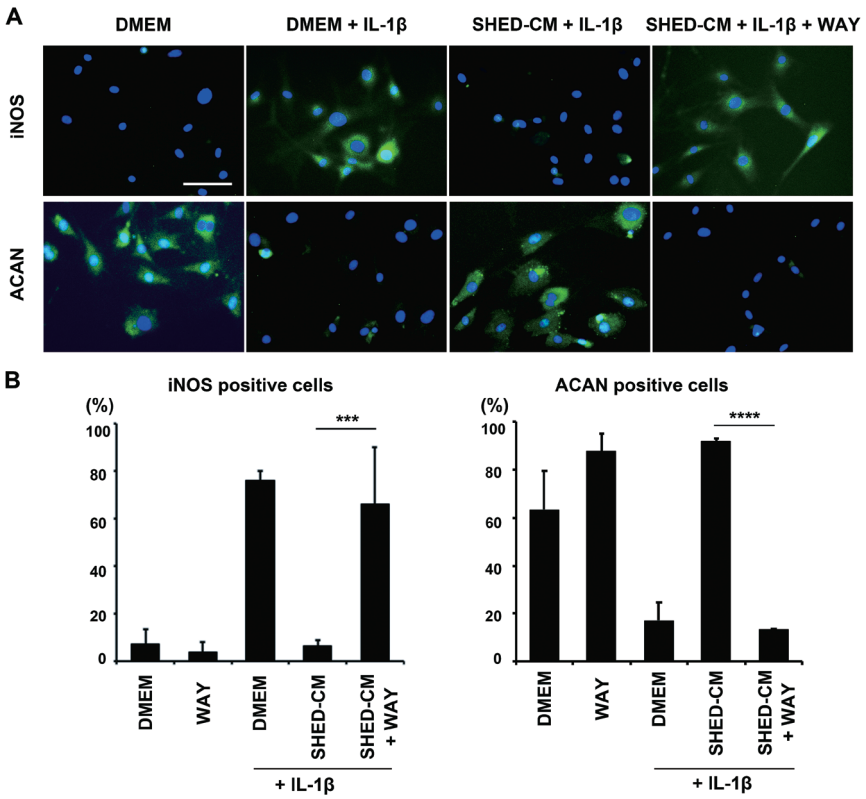

Fig. 3 Blocking of SFRP1 reduces the anti-inflammatory and pro-anabolic effects of SHED-CM.
(A) Representative immunofluorescence images.
(B) Percentage of iNOS-, and ACAN-positive chondrocytes in DAPI-positive. Similar results were obtained from two independent experiments.
Data represent mean ± SD. ***$p < 0.001$, ****$p < 0.0001$. Scale bar = 100 μm.

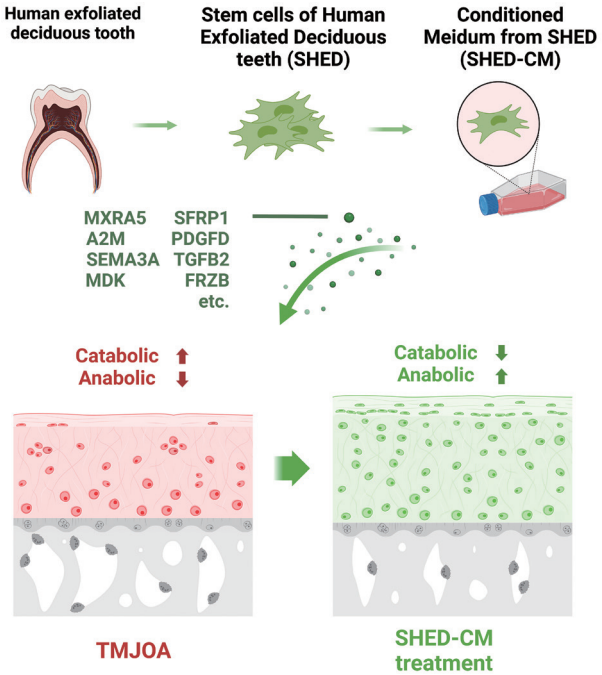

Fig. 4 Schematic diagram demonstrating the direct regulatory mechanism of SHED-CM in inflamed condylar chondrocytes.

and/or pro-chondrogenesis were identified: SFRP1, PDGFD, TGFB2, FRZB, MXRA5, A2M, SEMA3A, and MDK. PDGFD is a member of the PDGF family that enhances mitogenesis and chemotaxis of chondrocytes[23]. TGFβ maintains the homoeostasis of cartilage by promoting the differentiation of chondrocytes and biosynthesis of cartilage ECM components, collagen and proteoglycans[24, 25]. MXRA5, also known as adlican, is an adhesion proteoglycan that is highly expressed in the human OA synovial fluid[26, 27]. It has been reported that MXRA5 plays anti-inflammatory and anti-fibrotic functions in chronic inflammation, including in musculoskeletal diseases[28, 29]. A2M is a major protease inhibitor and can suppress catabolic activities in inflamed chondrocytes by blocking the IL-1β/NF-κB pathway, thus preventing cartilage injuries in OA[30, 31]. SEMA3A, which is a known osteoprotective factor, has also been shown to inhibit proinflammatory responses in lipopolysaccharide- or excessive stress-stimulated chondrocytes[32, 33]. MDK has been reported to promote the proliferation of articular chondrocytes, and knockdown of MDK in pre-chondrogenic ATDC5 cells results in the reduced expression of cartilage matrix-related proteins[34, 35].

Notably, among the eight target proteins, the Wnt signaling inhibitor SFPR1 was more abundant in SHED-CM than in BMSC-CM. Aberrant activation of Wnt/β-catenin signaling has been reported in OA cartilage[36, 37]. Activation of Wnt/β-catenin signaling by Wnt3A induces cartilage matrix degradation in IL-1β-stimulated chondrocytes[38]. Moreover, excessive stress- or IL-1β-induced catabolic activities are typically enhanced in chondrocytes isolated from mice lacking with other Wnt signaling inhibitors, such as FRZB $^{-/-}$ mice, compared to those in chondrocytes isolated from the wild type[39]. In the present study, we found that WAY-316606, a selective SFRP1 inhibitor, remarkably reduced the therapeutic effects of SHED-CM, which converted the catabolic phenotype to an anabolic phenotype in inflamed chondrocytes. These results demonstrated that SHED-CM partially restored the metabolic homeostasis of inflamed chondrocytes via SFRP1-mediated Wnt signaling inhibition. To the best of our knowledge, this study is the first to identify the therapeutic factor of SHED-CM, SFRP1, against the catabolic reaction in inflamed chondrocytes.

Conclusions

SHED-CM treatment directly suppressed catabolism and promoted the anabolic activities of inflamed primary condylar chondrocytes, in part through SFRP1. Further studies are needed to elucidate the therapeutic functions of other highly expressed proteins in SHED-CM. Our study suggest that SHED-CM may be useful for the treatment of inflammatory cartilage diseases.

Conflict of interest statement

The authors declare that they have no conflicts of interest in the present study.

Acknowledgments

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References

1) Wang XD, Zhang JN, Gan YH, Zhou YH: Current understanding of pathogenesis and treatment of TMJ osteoarthritis. J Dent Res 94, 666-673 (2015)
2) Tanaka E, Detamore MS, Mercuri LG: Degenerative disorders of the temporomandibular joint: etiology, diagnosis, and treatment. J Dent Res 87, 296-307 (2008)
3) Fang L, Ye Y, Tan X, Huang L, He Y: Overloading stress-induced progressive degeneration and self-repair in condylar cartilage. Ann N Y Acad Sci 1503, 72-87 (2021)
4) Kuroda S, Tanimoto K, Izawa K, Fujihara S, Koolstra JH, Tanaka E: Biomechanical and biochemical characteristics of the mandibular condylar cartilage. Osteoarthr Cartil 17, 1408-1415 (2009)
5) Li B, Guan G, Mei L, Jiao K, Li H: Pathological mechanism of chondrocytes and the surrounding environment during osteoarthritis of temporomandibular joint. J Cell Mol Med 25, 4902-4911 (2021)
6) Martel-Pelletier J, Barr AJ, Cicuttini FM, Conaghan PG, Cooper C, Goldring MB, Goldring SR, Jones G, Teichtahl AJ, Pelletier JP: Osteoarthritis. Nat Rev Dis Primers 2, 16072 (2016)
7) Barry F, Murphy M: Mesenchymal stem cells in joint disease and repair. Nat Rev Rheumatol 9, 584-594 (2013)
8) Cui D, Li H, Xu X, Ye L, Zhou X, Zheng L, Zhou Y: Mesenchymal stem cells for cartilage regeneration of TMJ osteoarthritis. Stem Cells Int 2017, 5979741 (2017)
9) Zhang R, Ma J, Han J, Zhang W, Ma J: Mesenchymal stem cell related therapies for cartilage lesions and osteoarthritis. Am J Transl Res 11, 6275-6289 (2019)
10) Barry F: MSC therapy for osteoarthritis: an unfinished story. J Orthop Res 37, 1229-1235 (2019)
11) Mancuso P, Raman S, Glynn A, Barry F, Murphy JM: Mesenchymal stem cell therapy for osteoarthritis:

the critical role of the cell secretome. Front Bioeng Biotechnol 7, 9 (2019)
12) Bousnaki M, Bakopoulou M, Kritis A, Koidis A: The efficacy of stem cells secretome application in osteoarthritis: a systematic review of in vivo studies. Stem Cell Rev Rep 16, 1222-1241 (2020)
13) Zhao Y, Xie L: An update on mesenchymal stem cell-centered therapies in temporomandibular joint osteoarthritis. Stem Cells Int 2021, 6619527 (2021)
14) Shi X, Mao J, Liu Y: Pulp stem cells derived from human permanent and deciduous teeth: Biological characteristics and therapeutic applications. Stem Cells Transl Med 9, 445-464 (2020)
15) Matsubara K, Matsushita Y, Sakai K, Kano F, Kondo M, Noda M, Hashimoto N, Imagama S, Ishiguro S, Suzumura A, Ueda M, Furukawa K, Yamamoto A: Secreted ectodomain of sialic acid-binding Ig-like lectin-9 and monocyte chemoattractant protein-1 promote recovery after rat spinal cord injury by altering macrophage polarity. J Neurosci 35, 2452-2464 (2015)
16) Ishikawa J, Takahashi N, Matsumoto T, Yoshioka Y, Yamamoto N, Nishikawa M, Hibi H, Ishigro N, Ueda M, Furukawa K, Yamamoto A: Factors secreted from dental pulp stem cells show multifaceted benefits for treating experimental rheumatoid arthritis. Bone 83, 210-219 (2016)
17) Liu Y, Kano F, Hashimoto N, Xia L, Zhou Q, Feng X, Hibi H, Miyazaki A, Iwamoto T, Matsuka Y, Zhang Z, Tanaka E, Yamamoto A: Conditioned medium from the stem cells of human exfoliated deciduous teeth ameliorates neuropathic pain in a partial sciatic nerve ligation model. Front Pharmacol 13, 745020 (2022)
18) Ogasawara N, Kano F, Hashimoto N, Mori H, Liu Y, Xia L, Sakamaki T, Hibi H, Iwamoto T, Tanaka E, Yamamoto A: Factors secreted from dental pulp stem cells show multifaceted benefits for treating experimental temporomandibular joint osteoarthritis. Osteoarthr Cartil 28, 831-841 (2020)
19) Sakai K, Yamamoto A, Matsubara K, Nakamura S, Naruse M, Yamagata M, Sakamoto K, Tauchi R, Wakao N, Imagama S, Hibi H, Kadomatsu K, Ishiguro N, Ueda M: Human dental pulp-derived stem cells promote locomotor recovery after complete transection of the rat spinal cord by multiple neuro-regenerative mechanisms. J Clin Investig 122, 80-90 (2012)
20) Gosset M, Berenbaum F, Thirion F, Jacques C: Primary culture and phenotyping of murine chondrocytes. Nat Protoc 3, 1253-1260 (2008)
21) Motani K, Kosako H: Activation of stimulator of interferon genes (STING) induces ADAM17-mediated shedding of the immune semaphorin SEMA4D. J Biol Chem 293, 7717-7726 (2018)
22) Wang Y, Fan X, Xing L, Tian F: Wnt signaling: a promising target for osteoarthritis therapy. Cell Commun Signal 17, 97 (2019)
23) Schmidt MB, Chen EH, Lynch SE: A review of the effects of insulin-like growth factor and platelet derived growth factor on in vivo cartilage healing and repair. Osteoarthr Cartil 14, 403-412 (2006)
24) Patil AS, Sable RB, Kothari RM: An update on transforming growth factor-b (TGF-b): sources, types, functions and clinical applicability for cartilage/bone healing. J Cell Physiol 226, 3094-3103 (2011)
25) Xu X, Zheng L, Yuan Q, Zhen G, Crane JL, Zhou X, Cao X: Transforming growth factor-β in stem cells and tissue homeostasis. Bone Res 6, 2 (2018)
26) Walker MG, Volkmuth W: Cell adhesion and matrix remodeling genes identified by co-expression analysis. Gene Funct Dis 3, 109-112 (2002)
27) Balakrishnan L, Nirujogi RS, Ahmad RS, Bhattacharjee M, Manda SS, Renuse S, Kelkar DS, Subbannayya Y, Raju R, Goel R, Thomas JK, Kaur N, Dhillon M, Tankala SG, Jois R, Vasdev V, Ramachandra YL, Sahasrabuddhe NA, Prasad TSK, Mohan S, Gowda H, Shankar S, Pandey A: Proteomic analysis of human osteoarthritis synovial fluid. Clin Proteom 11, 6 (2014)
28) Poveda J, Sanz AB, Fernandez-Fernandez B, Carrasco S, Ruiz-Ortega M, Cannata-Ortiz P, Ortiz A, Sanchez-Niño MD: MXRA5 is a TGF-β1-regulated human protein with anti-inflammatory and anti-fibrotic properties. J Cell Mol Med 21, 154-164 (2017)
29) Mocchi M, Grolli S, Dotti S, Di Silvestre D, Villa R, Berni P, Conti V, Passignani G, Brambilla F, Bue MD, Catenacci L, Sorrenti M, Segale L, Bari E, Mauri P, Torre ML, Perteghella S: Equine mesenchymal stem/stromal cells freeze-dried secretome (lyosecretome) for the treatment of musculoskeletal diseases: production process validation and batch release test for clinical use. Pharmaceuticals 14, 553 (2021)
30) Sun C, Cao C, Zhao T, Guo H, Fleming BC, Owens B, Beveridge J, McAllister S, Wei L: A2M inhibits inflammatory mediators of chondrocytes by blocking IL-1β/NF-κB pathway. J Orthop Res 41, 241-248 (2022)
31) Cuéllar JM, Cuéllar VG, Scuderi GJ: α2-Macroglobulin: autologous protease inhibition technology. Phys Med Rehabil Clin N Am 27, 909-918 (2016)
32) Sumi C, Hirose N, Yanoshita M, Takano M, Nishiyama S, Okamoto Y, Asakawa Y, Tanimoto K: Semaphorin 3A inhibits inflammation in chondrocytes under excessive mechanical stress. Mediators Inflamm 2018, 5703651

(2018)

33）Zhang H, Lu Y, Wu B, Xia F: Semaphorin 3A mitigates lipopolysaccharide-induced chondrocyte inflammation, apoptosis and extracellular matrix degradation by binding to Neuropilin-1. Bioengineered 12, 9641-9654 (2021)

34）Zhang ZH, Li HX, Qi YP, Du LJ, Zhu SY, Wu MY, Lu HL, Yu Y, Han Y: Recombinant human midkine stimulates proliferation of articular chondrocytes. Cell Prolif 43, 184-194 (2010)

35）Haffner-Luntzer M, Heilmann A, Rapp AE, Beie S, Schinke S, Amling M, Ignatius A, Liedert A: Midkine-deficiency delays chondrogenesis during the early phase of fracture healing in mice. PLOS ONE 9, e116282 (2014)

36）Zhu M, Tang D, Wu Q, Hao S, Chen M, Xie C, Rosier RN, O'Keefe RJ, Zuscik M, Chen D: Activation of β-catenin signaling in articular chondrocytes leads to osteoarthritis-like phenotype in adult β-catenin conditional activation mice. J Bone Miner Res 24, 12-21 (2009)

37）Usam Y i, Gunawardena AT, Iwamoto M, Enomoto-Iwamoto M: Wnt signaling in cartilage development and diseases: lessons from animal studies. Lab Investig 96, 186-196 (2016)

38）Yuasa T, Otani T, Koike T, Iwamoto M, Enomoto-Iwamoto M: Wnt/β-catenin signaling stimulates matrix catabolic genes and activity in articular chondrocytes: its possible role in joint degeneration. Lab Investig 88, 264-274 (2008)

39）Bougault M, Priam S, Houard X, Pigenet A, Sudre L, Lories RJ, Jacques C, Berenbaum F: Protective role of frizzled-related protein B on matrix metalloproteinase induction in mouse chondrocytes. Arthritis Res Ther 16, R137 (2014)