Research Article

Proteomic Analysis Reveals Commonly Secreted Proteins of Mesenchymal Stem Cells Derived from Bone Marrow, Adipose Tissue, and Synovial Membrane to Show Potential for Cartilage Regeneration in Knee Osteoarthritis

Yura Lee,1 Yo Seph Park,1 Na Young Choi,1 Yong Il Kim,1 and Yong-Gon Koh2

1Department of Stem Cell Research, Research and Development Center, TJC Life, Seoul 08502, Republic of Korea
2Department of Orthopaedic Surgery, Yonsei Sarang Hospital, Seoul 06698, Republic of Korea

Correspondence should be addressed to Yong-Gon Koh; osygkoh@gmail.com

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Paracrine factors secreted by mesenchymal stem cells (MSCs) reportedly modulate inflammation and reparative processes in damaged tissues and have been explored for knee osteoarthritis (OA) therapy. Although various studies have reported the effects of paracrine factors in knee OA, it is not yet clear which paracrine factors directly affect the regeneration of damaged cartilage and which are secreted under various knee OA conditions. In this study, we cultured MSCs derived from three types of tissues and treated each type with IL-1β and TNF-α or not to obtain conditioned medium. Each conditioned medium was used to analyse the paracrine factors related to cartilage regeneration using liquid chromatography-tandem mass spectrometry. Bone marrow-, adipose tissue-, and synovial membrane-MSCs (all-MSCs) exhibited expression of 93 proteins under normal conditions and 105 proteins under inflammatory conditions. It was confirmed that the types of secreted proteins differed depending on the environmental conditions, and the proteins were validated using ELISA. The results of Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analysis using a list of proteins secreted by all-MSCs under each condition confirmed that the secreted proteins were closely related to cartilage repair under inflammatory conditions. Protein-protein interaction networks were confirmed to change depending on environmental differences and were found to enhance the secretion of paracrine factors related to cartilage regeneration under inflammatory conditions. In conclusion, our results demonstrated that compared with knee OA conditions, the differential expression proteins may contribute to the regeneration of damaged cartilage. In addition, the detailed information on commonly secreted proteins by all-MSCs provides a comprehensive basis for understanding the potential of paracrine factors to influence tissue repair and regeneration in knee OA.

1. Introduction

Mesenchymal stem cells (MSCs) can be easily obtained from different cell sources such as bone marrow (BM), adipose tissue (AT), and synovial membrane (SM). They have self-renewal and trilineage differentiation potential [1–4]. MSCs are also known to secrete various paracrine factors including cytokines, chemokines, growth factors, and extracellular vesicles. Paracrine signalling is a form of cell-to-cell communication in which a cell produces a signal to induce changes in nearby cells, altering the behaviour or differentiation of those cells. Paracrine factors secreted by MSCs induce surrounding cells to differentiate into mature cell lines and regulate tissue inflammation or recovery processes [5]. Thus, there is increasing evidence that MSCs play a role in regenerating damaged tissue through the paracrine effect on surrounding cells [2, 6, 7]. Owing to these characteristics, MSCs are widely studied for the treatment for various diseases. According to the official database of ClinicalTrials.gov, 630 MSC-based clinical trials have been reported, and 10% or more of them are actively being conducted to assess the potential of MSCs for knee osteoarthritis (OA) therapy.

Knee OA is a degenerative disease caused by various factors, including abnormal mechanical stress, ageing, obesity,
and genetic factors, and results in inflammation in joints and degradation of cartilage tissue [8]. The cartilage of the knee joint where knee OA occurs is difficult to regenerate after injury due to the nature of cartilage; therefore, therapeutic treatments are limited to conservative care or artificial joint surgery [9, 10]. To overcome these therapeutic limitations and augment cartilage regeneration, various tissue-derived MSCs are being used for the development of cell therapy for knee OA treatment [11–14]. According to the results of recent studies, injecting MSC-conditioned medium (CM) into the anterior cruciate ligament transection rat model is sufficient to mediate cartilage regeneration function [11, 15–17]; thus, interest in efficacious knee OA treatments using MSC-derived paracrine factors has increased [14, 18–21]. However, although several studies have actively investigated paracrine factors secreted by each type of MSC obtained from various tissues under general culture conditions [22–24], it is still unclear which paracrine factors affect the regeneration of damaged cartilage.

To elucidate the therapeutic role of paracrine factors secreted by MSCs, it is necessary to identify the factors that are commonly secreted by MSCs under various environmental conditions, since each type of tissue-derived MSC secretes different paracrine factors [24–26]. However, little is known about how environmental conditions similar to knee OA affect paracrine factors [27, 28]. Therefore, in the present study, we aimed to investigate the major paracrine factors related to cartilage regeneration by identifying the differences in the types and characteristics of commonly secreted proteins by BM-, AT-, and SM-MSCs under various conditions.

2. Materials and Methods

2.1. Isolation and Culture of Human BM-, AT-, and SM-MSCs. The study was approved by the Institutional Review Board of Yonsei Sarang Hospital (IRB number: YSSR 2020-09-001), and informed consent was obtained from all donors. Human BM-MSCs (n = 3) were purchased from the American Type Culture Collection (Manassas, VA, USA). The frozen cells were thawed and plated at a density of 5,000 cells/cm² in a 75 cm² culture flask with a complete medium containing alpha modification of Eagle’s medium (α-MEM; Welgene, Daegu, Republic of Korea), 10% foetal bovine serum (FBS; HyClone, Logan, UT, USA), and 1% penicillin-streptomycin (P/S; HyClone).

Human AT (n = 3) were harvested by liposuction surgery. The tissue was digested with 0.3% collagenase type 1 ( Worthington-Biochemical, Lakewood, NJ, USA) at 37°C for 90 min with gentle shaking. After digestion, the digested tissues were washed with phosphate-buffered saline (PBS;
HyClone) and the undigested tissues were removed using a 70 μm strainer (BD Biosciences, San Diego, CA, USA). The cells obtained by centrifugation at \(645 \times g\) for 5 min were plated at a density of 5,000 cells/cm\(^2\) in a 75 cm\(^2\) culture flask with complete medium [29].

Human SM (\(n = 3\)) were harvested from the knees of patients with OA during total knee arthroplasty. The tissue was placed on a petri dish and finely minced using sterile scissors. The minced tissue was digested with 0.25% Trypsin-EDTA (HyClone) at 37°C for 30 min. After digestion, 0.3% of collagenase type 1 was added and gently shaken at 37°C for 90 min. Undigested tissues were removed using a 40 μm strainer (BD Biosciences), and cells were obtained by centrifugation at \(645 \times g\) for 5 min. The obtained cells were plated at a density of 5,000 cells/cm\(^2\) in a 75 cm\(^2\) culture flask with complete medium [29, 30].

All cells used in the experiment were cultured in a humidified incubator at 5% CO\(_2\) at 37°C. The medium was changed every 2–3 days, and cells were subcultured at 80–90% confluence following treatment with 0.25% Trypsin-EDTA at 37°C for 5 min. The cells were washed and harvested by centrifugation at \(645 \times g\) for 5 min and then plated at a density of 5,000 cells/cm\(^2\) [32, 33]. Passage 4 cells were used for all cell types.

### 2.2. Flow Cytometry

For flow cytometry analysis, \(1 \times 10^5\) cells were suspended in 100 μL of PBS [33]. The following monoclonal antibodies were used to stain the cells: fluorescein isothiocyanate (FITC) mouse anti-human CD14 (clone M5E2), FITC mouse anti-human CD34 (clone 581), FITC mouse anti-human CD45 (clone HI30), phycoerythrin (PE) mouse anti-human CD79a (clone HM47), and FITC mouse anti-human leukocyte antigen-DR (HLA-DR; clone G46-6) were used as haematopoietic or endothelial cell markers. PerCP-Cy5.5 mouse anti-human CD73 (clone AD2), PerCP-Cy5.5 mouse anti-human CD90 (clone 5E10), and PerCP-Cy5.5 mouse anti-human CD105 (clone 266) were used as MSC-specific markers. As isotype controls, FITC mouse IgG1 κ (clone MOPC-21), FITC mouse IgG2a κ (clone G155-178), PE mouse IgG1 κ (clone MOPC-21), and PerCP-Cy5.5 mouse IgG1 κ (clone MOPC-21; all from BD Biosciences) were used [34]. Stained cells were acquired using FACSCalibur (BD Biosciences), and data analysis was performed using CellQuest software (BD Biosciences).
Table 1: The list of commonly secreted proteins identified by all mesenchymal stem cells (MSCs) under normal and inflammatory conditions.

(a) Identified proteins of normal condition (105 proteins)

| Accession | Description | Gene symbol |
|-----------|-------------|-------------|
| A0A024QYT5 | Serpin peptidase inhibitor, clade E | SERPINE1 |
| A0A024R1U8 | Insulin-like growth factor-binding protein 4 | IGFBP4 |
| A0A024R2W4 | Dystroglycan 1 | DAG1 |
| A0A024R462 | Fibronectin 1 | FN1 |
| A0A024R6R4 | Matrix metallopeptidase 2 | MMP2 |
| A0A024R8V7 | TIMP metallopeptidase inhibitor 2 | TIMP2 |
| A0A024RDW8 | Collagen, type IV, alpha 2 | COL4A2 |
| A0A087WTA8 | Collagen alpha-2(I) chain | COL1A2 |
| A0A087X0S5 | Collagen alpha-1(VI) chain | COL6A1 |
| A0A0A0MT01 | Gelsolin | GSN |
| A0A0F7G8J1 | Plasminogen | PLG |
| A0A140VJI7 | Testicular tissue protein Li 61 | ECM1 |
| A0A161I202 | Lactoferrin | LTF |
| A0A172Q381 | Endosialin | CD248 |
| A1L4H1 | Soluble scavenger receptor cysteine-rich domain-containing protein SSC5D | SSC5D |
| A4D2D2 | Procollagen C-endopeptidase enhancer | PCOLCE |
| A6XND1 | Insulin-like growth factor-binding protein 3 | IGFBP3 |
| A8K2H4 | Cathepsin B | CTSB |
| A8K7Q1 | Nucleobindin 1 | NUCB1 |
| A8KAJ3 | EGF-containing fibulin-like extracellular matrix protein 1 | EFEMP1 |
| B2R582 | C-type lectin domain family 3, member B | CLEC3B |
| B2R5M9 | Procollagen-lysine, 2-oxoglutarate 5-dioxygenase | PLOD1 |
| B2RB8 | Albumin | ALB |
| B2RCM5 | EG-containing fibulin-like extracellular matrix protein 2 | EFEMP2 |
| B2RDW0 | Calmodulin 2 (phosphorylase kinase, delta) | CALM2 |
| B4DDQ2 | Biglycan | BGN |
| B4DNG0 | Olfactomedin-like protein 3 | OLFML3 |
| B4DPH4 | Plasminogen | PLG |
| B4DPQ0 | Complement C1r subcomponent | C1R |
| B4DPZ5 | Polymerase I and transcript release factor | PTRF |
| B4DU16 | Fibronectin 1 | FN1 |
| B4E3Q1 | Calsyntenin-1 | CLSTN1 |
| D0PN1 | Lysyl oxidase | LOX |
| D1MGQ2 | Alpha-2 globin chain | HBA2 |
| D3DTX7 | Collagen, type I, alpha 1 | COL1A1 |
| D3YTG3 | Target of Nesh-SH3 | ABI3BP |
| D6RF35 | Vitamin D-binding protein | GC |
| D9ZGG2 | Vitronectin | VTN |
| F8VR42 | Dynein regulatory complex subunit 2 | CCDC65 |
| F8W6I7 | Heterogeneous nuclear ribonucleoprotein A1 | HNRNP A1 |
| H0YGS3 | Microfibrillar-associated protein 5 | MFAP5 |
| H7BJ3 | Protein disulfide-isomerase A3 | PDI A3 |
| H7C0V9 | Amyloid-beta A4 protein | APP |
| I4AY87 | Macrophage migration inhibitory factor | MIF |
| O00391 | Sulphydryl oxidase 1 | QSOX1 |
Table 1: Continued.

| Accession | Description | Gene symbol |
|-----------|-------------|-------------|
| P00338    | L-Lactate dehydrogenase A chain | LDHA        |
| P00441    | Superoxide dismutase [Cu-Zn] | SOD1        |
| P01023    | Alpha-2-macroglobulin | A2M         |
| P01024    | Complement C3 | C3          |
| P01033    | Metalloprotease inhibitor 1 | TIMP1       |
| P01034    | Cystatin-C | CST3        |
| P02452    | Collagen alpha-1(I) chain | COL1A1      |
| P02461    | Collagen alpha-1(III) chain | COL3A1      |
| P02647    | Apolipoprotein A-I | APOA1       |
| P05387    | 60S acidic ribosomal protein P2 | RPLP2      |
| P05997    | Collagen alpha-2(V) chain | COL5A2      |
| P07585    | Decorin | DCN         |
| P07737    | Profilin-1 | PFN1        |
| P07942    | Laminin subunit beta-1 | LAMB1       |
| P08670    | Vimentin | VIM         |
| P09382    | Galectin-1 | LGALS1      |
| P09486    | SPARC | SPARC       |
| P09871    | Complement C1s subcomponent | C1S         |
| P10599    | Thioredoxin | TXN         |
| P11047    | Laminin subunit gamma-1 | LAMC1      |
| P12110    | Collagen alpha-2(VI) chain | COL6A2      |
| P14543    | Nidogen-1 | NID1        |
| P18206    | Vinculin | VCL         |
| P21333    | Filamin-A | FLNA        |
| P24592    | Insulin-like growth factor-binding protein 6 | IGFBP6     |
| P26022    | Pentraxin-related protein PTX3 | PTX3       |
| P30041    | Peroxiredoxin-6 | PRDX6       |
| P35442    | Thrombospondin-2 | THBS2      |
| P35555    | Fibrillin-1 | FBN1        |
| P36955    | Pigment epithelium-derived factor | SERPINF1   |
| P48061    | Stromal cell-derived factor 1 | CXCL12     |
| P50454    | Serpin H1 | SERPINH1    |
| P51884    | Lumican | LUM         |
| P55290    | Cadherin-13 | CDH13      |
| P63261    | Actin, cytoplasmic 2 | ACTG1      |
| P63313    | Thymosin beta-10 | TMSB10     |
| Q01995    | Transgelin | TAGLN       |
| Q08629    | Testican-1 | SPOCK1      |
| Q02944    | Beta globin | HBB         |
| Q12841    | Follistatin-related protein 1 | FSTL1      |
| Q14767    | Latent-transforming growth factor beta-binding protein 2 | LTBP2     |
| Q15582    | Transforming growth factor-beta-induced protein ig-h3 1 | TGFBI     |
| Q16270    | Insulin-like growth factor-binding protein 7 | IGFBP7    |
| Q16778    | Histone H2B type 2-E | HIST2H2BE  |
| Q53FA4    | Cysteine-rich, angiogenic inducer, 61 variant | CYR61      |
| Q53G99    | Beta actin variant | ACTB        |
| Q59GA0    | Thy-1 cell surface antigen variant | THY1       |
| Q5M8T4    | Connective tissue growth factor | CTGF        |
### Table 1: Continued.

#### (a) Identified proteins of normal condition (105 proteins)

| Accession | Description                          | Gene symbol |
|-----------|--------------------------------------|-------------|
| Q6EMK4    | Vasorin                              | VASN        |
| Q6HC9     | STC2 protein                         | STC2        |
| Q6HW3     | DF protein                           | CFD         |
| Q6IAW5    | CALU protein                         | CALU        |
| Q6HK3     | CD109 antigen                        | CD109       |
| Q8IUX7    | Adipocyte enhancer-binding protein 1 | AEBP1       |
| Q99715    | Collagen alpha-1(XII) chain          | COL12A1     |
| V9HWc6    | Peptidyl-prolyl cis-trans isomerase  | PPIB        |
| V9HEW8    | Epididymis secretory sperm binding protein Li 47e | ARHGDIA |
| V9HW15    | Coflin 1 (nonmuscle)                 | CFL1        |
| V9HWK1    | Triosephosphate isomerase            | TP1         |
| V9HWN7    | Fructose-bisphosphate aldolase       | ALDOA       |

#### (b) Identified proteins of inflammatory condition (93 proteins)

| Accession | Description                                      | Gene symbol |
|-----------|--------------------------------------------------|-------------|
| A0A024R1U8 | Insulin-like growth factor binding protein 4 | IGFBP4      |
| A0A024R462 | Fibronectin 1                                   | FN1         |
| A0A024R5Z7 | Annexin                                         | ANXA2       |
| A0A024R6R4 | Matrix metallopeptidase 2                       | MMP2        |
| A0A024R8V7 | TIMP metallopeptidase inhibitor 2               | TIMP2       |
| A0A024R969 | Chitinase 3-like 1                              | CHI3L1      |
| A0A024RDA5 | Multifunctional fusion protein                  | IL-8; CXCL8 |
| A0A087WTA8 | Collagen alpha-2(I) chain                       | COL1A2      |
| A0A087X0S5 | Collagen alpha-1(VI) chain                     | COL1A6      |
| A0A0A0MT01 | Gelsolin                                        | GSN         |
| A0A0S2ZJG9 | Actinin alpha 4 isoform 1                       | ACTN4       |
| A0A1611202 | Lactoferrin                                     | LTF         |
| A0A1B0G9U2 | Uncharacterized protein                         | N/A         |
| A0A1U9X7H4 | CFB                                              | CFB         |
| A4D1W7    | Inhibin, beta A (activin A, activin AB alpha polypeptide) | INHBA |
| A4D2D2    | Procollagen C-endopeptidase enhancer            | PCOLCE      |
| A6XND1    | Insulin-like growth factor binding protein 3 isoform b | IGFBP3 |
| A8K7Q1    | Nucleobindin 1                                  | NUCB1       |
| B2R4R0    | Histone H4                                      | HIST1H4A    |
| B2R5J8    | C-C motif chemokine                             | CCL5        |
| B2RBS8    | Albumin                                         | ALB         |
| B2RCM5    | EGF-containing fibulin-like extracellular matrix protein 2 | EFEMP2 |
| B2RDW0    | Calmodulin 2                                    | CALM2       |
| B3KQT9    | Protein disulfide-isomerase                     | PDIA3       |
| B4DQQ2    | Biglycan                                        | BGN         |
| B4DLV7    | Rab GDP dissociation inhibitor                  | GDI2        |
| B4DMR3    | Gla-derived nexin                              | SERPINE2    |
| B4DPQ0    | Complement C1r subcomponent                    | C1R         |
| B4E324    | Lysosomal protective protein                    | CTSA        |
| B4EQQ1    | Calsyntenin-1                                   | CLSTN1      |
| B5MCZ3    | Interleukin-6                                   | IL-6        |
| Accession | Description | Gene symbol |
|-----------|-------------|-------------|
| D0PN1     | Epididymis luminal protein 4 | YWHAZ       |
| D3D7X7    | Collagen, type I, alpha 1 | COL1A1      |
| D3YTG3    | Target of Nesh-SH3 | AB13BP      |
| D6RF92    | C-X-C motif chemokine | CXCL6       |
| D9ZGF2    | Collagen, type VI, alpha 3 | COL6A3      |
| F8W67     | Heterogeneous nuclear ribonucleoprotein A1 | HNRNPA1 |
| I4AY87    | Macrophage migration inhibitory factor | MIF |
| O00300    | Tumor necrosis factor receptor superfamily member 11B | TNFRSF11B |
| O00391    | Sulphhydril oxidase 1 | QSOX1       |
| P01024    | Complement C3 | C3          |
| P01033    | Metalloproteinase inhibitor 1 | TIMP1      |
| P01034    | Cystatin-C | CST3        |
| P02461    | Collagen alpha-1(III) chain | COL3A1     |
| P04083    | Annexin A1 | ANXA1       |
| P07585    | Decorin | DCN         |
| P07737    | Profilin-1 | PFN1        |
| P07996    | Thrombospondin-1 | THBS1 |
| P08254    | Stromelysin-1 | MMP3 |
| P08670    | Vimentin | VIM         |
| P09341    | Growth-regulated alpha protein | CXCL1     |
| P09382    | Galectin-1 | LGALS1     |
| P09486    | SPARC | SPARC       |
| P09871    | Complement C1s subcomponent | C1S |
| P10599    | Thioredoxin | TXN |
| P11047    | Laminin subunit gamma-1 | LAMC1      |
| P12110    | Collagen alpha-2(VI) chain | COL6A2     |
| P13500    | C-C motif chemokine 2 | CCL2       |
| P14543    | Nidogen-1 | NID1        |
| P15018    | Leukemia inhibitory factor | LIF        |
| P18206    | Vinculin | VCL         |
| P19875    | C-X-C motif chemokine 2 | CXCL2      |
| P19876    | C-X-C motif chemokine 3 | CXCL3      |
| P20809    | Interleukin-11 | IL-11      |
| P21333    | Filamin-A | FLNA        |
| P24592    | Insulin-like growth factor-binding protein 6 | IGFBP6 |
| P26022    | Pentraxin-related protein PTX3 | PTX3      |
| P35442    | Thrombospondin-2 | THBS2 |
| P35555    | Fibrillin-1 | FBN1       |
| P48307    | Tissue factor pathway inhibitor 2 | TFPI2     |
| P51884    | Lumican | LUM         |
| P62328    | Thymosin beta-4 | TMSB4X |
| P63313    | Thymosin beta-10 | TMSB10 |
| P98066    | Tumor necrosis factor-inducible gene 6 protein | TNFAIP6; TSG-6 |
| Q12841    | Follistatin-related protein 1 | FSTL1      |
| Q16270    | Insulin-like growth factor-binding protein 7 | IGFBP7 |
| Q16778    | Histone H2B type 2-E | HIST2H2BE |
| Q53G71    | Calreticulin variant | CALR |
| Q53G75    | Matrix metalloproteinase 1 preproprotein variant | MMP1 |
2.3. In Vitro Differentiation Assay. For adipogenic differentiation, cells were seeded into 12-well plates at a density of $3 \times 10^4$ per well. After 2 days, differentiation was induced using adipogenic differentiation medium (containing $\alpha$-MEM, 10% FBS, 1% P/S, 0.5 mM of 3-isobutyl-1-methylxanthine (Sigma-Aldrich, St. Louis, MO, USA), 1 $\mu$M of dexamethasone (Sigma-Aldrich), 200 $\mu$M of indomethacin (Sigma-Aldrich), and 10 $\mu$g/mL of insulin (Gibco, Waltham, Massachusetts, USA)) [35]. The medium was changed every 2–3 days for 14 days, and differentiated cells were stained with Oil Red O (Sigma-Aldrich) according to the manufacturer’s instructions.

For osteogenic differentiation, cells were seeded into 12-well plates at a density of $3 \times 10^4$ per well. After 2 days, differentiation was induced using osteogenic differentiation medium (containing $\alpha$-MEM, 10% FBS, 1% P/S, 0.1 $\mu$M of dexamethasone, 50 $\mu$M of L-ascorbic acid 2-phosphate sequinagens salt hydrate (Sigma-Aldrich), and 20 $\mu$M of $\beta$-glycerophosphate disodium salt hydrate (Sigma-Aldrich)) [36]. The medium was changed every 2–3 days for 14 days, and differentiated cells were stained with Alizarin Red S (IHC World, Woodstock, MD, USA) according to the manufacturer’s instructions.

For chondrogenic differentiation, cells were seeded into 12-well plates at a density of $1.2 \times 10^4$ per well. After 2 days, differentiation was induced using chondrogenic differentiation medium (containing Dulbecco’s minimal EM-high glucose (HyClone), 10% FBS, 1% P/S, 0.1 $\mu$M of dexamethasone, 1X insulin-transferrin-selenium (Gibco), 50 $\mu$M of L-ascorbic acid 2-phosphate sequinagens salt hydrate, and 5 ng/mL of transforming growth factor $\beta 1$ (TGF- $\beta 1$; PeproTech, Rocky Hill, NJ, USA)) [37]. The medium was changed every 2–3 days for 21 days, and differentiated cells were stained with Alcian Blue 8GX (Sigma-Aldrich) according to the manufacturer’s instructions.

2.4. Preparation of CM. The BM-, AT-, and SM-MSCs were maintained under two conditions based on environmental differences: (i) normal condition: without inflammatory cytokines (tumour necrosis factor-$\alpha$ (TNF-$\alpha$; PeproTech) and interleukin-1$\beta$ (IL-1$\beta$; PeproTech)), and (ii) inflammatory condition: treated with 10 ng/mL of TNF-$\alpha$ and 10 ng/mL of IL-1$\beta$ [38–41]. To prepare CM, cells were cultured in a 75 cm$^2$ culture flask in $\alpha$-MEM containing 10% FBS. When MSCs were approximately 80–90% confluent at passage 4, the cells were switched to serum-free $\alpha$-MEM for overnight incubation and treated with conditions (i) or (ii) for 6h. Cultures were refed with 15 mL of serum-free $\alpha$-MEM and incubated for 48 h. Here, we used serum-free medium to avoid interference from albumin-enriched FBS. Then, the CM was collected and filtrated through a 0.2 $\mu$m filter to remove cellular debris. Thereafter, the CM was concentrated 25-fold using ultrafiltration units with 3 kDa cutoff filters (Amicon Ultra; Merck Millipore, Watford, UK) at 4000 $\times$ g for 1 h [42]. The protein concentration of the CM was measured using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer’s instructions.

2.5. In-Solution Digestion. Briefly, 100–200 $\mu$g of protein was denatured using 8 M urea in 50 mM heavy carbonate ammonium buffer (pH 7.8) and allowed to react at room temperature for 3 h, followed by reduction using 10 mM dithiothreitol for 2 h at room temperature. The proteins were alkylated with 10 mM iodoacetamide in the dark at room temperature for 1 h and then diluted more than 10-fold using 50 mM ammonium bicarbonate solution. In-solution digestion was carried out by adding trypsin to the protein solution with an enzyme-to-protein ratio of 1:50 ($w/w$) at 37°C for 18 h. Finally, formic acid was added to each sample to stop the reaction. Samples were stored at -80°C until further analysis [43].

2.6. Liquid Chromatography with Tandem Mass Spectrometry. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis was conducted by the National Instrumentation Center for Environmental Management (NICEM;
Seoul National University, Seoul, Republic of Korea). The fractionated peptide samples were analysed using the LC-MS/MS system, which was a combination of an Easy-nLC 1000 (Thermo Fisher Scientific) and EASY-Spray Ion Source (Thermo Fisher Scientific) on a Q Exactive Orbitrap Mass Spectrometer (Thermo Fisher Scientific). Peptides were separated on a two-column setup with a trap column (Thermo Fisher Scientific Acclaim PepMap 100 C18 HPLC Columns; 100 μm x 2 cm, nanoViper C18, particle size of 5 μm; Thermo Fisher Scientific, part number 164564) and an analytical column (Thermo Fisher Scientific; 75 μm i.d. x 50 cm long, 2 μm C18 beads, spherical, fully porous, ultrasphere). The peptide samples were separated using a 180 min linear gradient from 10% to 40% solvent B (100% acetonitrile and 0.1% formic acid) in all samples. The spray voltage was 2.2 kV in a positive ion mode, and the temperature of the heated capillary was set to 300°C. Mass spectra were acquired in a data-dependent manner using the top ten methods on the Q Exactive Mass Spectrometer. Xcalibur software version 3.1 was used to collect MS data. The Orbitrap analyser scanned precursor ions with a mass range of 350–1,800 m/z with a resolution of 70,000 at m/z 200. The automatic gain control (AGC) target value was 3 x 10^6, and the isolation window for MS/MS was 2 m/z. Higher-energy C-trap dissociation scans were acquired at a resolution of 17,500 and normalised collision energy of 27. The AGC target value for MS/MS was 1 x 10^5. The maximum ion injection time for the survey scan and MS/MS scan was 100 ms. Dynamic exclusion was enabled with an exclusion period of 15 s [44].

Mass data were acquired automatically using MaxQuant version 1.6 and Proteome Discoverer 2.3 (Thermo Fisher Scientific). LC-MS/MS analysis was performed thrice on the samples (triplicates for each MSC type under normal or inflammatory conditions).

2.7. Bioinformatics Analysis. The original MS/MS file data were acquired automatically with Proteome Discoverer 2.3 (version 2.3.0.523) for data analysis. Peptides were identified using SEQUEST-HT against the UniProtKB database (uniprot-homosapiens-201810) integrated into Proteome Discoverer. The processing workflow consisted of the following nodes: two maximum missed cleavages, peptide length range of 6–144 amino acids, precursor mass tolerance of 10 ppm, fragment mass tolerance of 0.02 Da, cysteine carbamidomethylation as a static modification, and oxidation as a dynamic modification. Peptide validation settings were identified using a target false discovery rate (FDR; strict) for peptide-spectrum match (PSM) of 0.01, target FDR (relaxed) for PSM of 0.05, and peptide filter confidence of at least high level.

The identified proteins were associated with Gene Ontology (GO) terms to determine their biological and functional properties. The three main types of annotations, namely, cellular components (CC), molecular functions (MF), and biological processes (BP), were obtained from the GO website at http://www.geneontology.org.

Protein pathways were generated using Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. In addition, the identified proteins were entered into the STRING database (https://string-db.org/) to predict and visualise the protein-protein interactions (PPI) under various environmental conditions. A representative network was obtained with high confidence in data settings with a minimum interaction score of 0.7.

2.8. Enzyme-Linked Immunosorbent Assay. Cell culture supernatants were collected as described in the preparation of CM. The concentration of proteins in CM was assessed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (RayBiotech, GA, USA) according to the manufacturer’s instructions [45]. Assay kits for the following proteins were used: human thrombospondin 2 (TSP-2) and human TNF-inducible gene 6 (TSG-6). All measurements were performed in duplicate.

The figure shows the Gene Ontology (GO) annotation analysis of the commonly secreted proteins by all mesenchymal stem cells (MSCs) under normal and inflammatory conditions: (a) molecular function; (b) cellular component; (c) biological process.

**Figure 3: Gene Ontology (GO) annotation analysis of the commonly secreted proteins by all mesenchymal stem cells (MSCs) under normal and inflammatory conditions: (a) molecular function; (b) cellular component; (c) biological process.**
3. Results

3.1. Characterization of BM-, AT-, and SM-MSCs. The human BM-, AT-, and SM-MSCs derived from each tissue were found to grow in a spindle-shaped manner (Figure 1(a)). We performed in vitro differentiation assays to confirm the trilineage differentiation potential of each MSC type. As a result, each MSC type differentiated into adipocytes, osteoblasts, and chondrocytes when cultured in the appropriate differentiation medium (Figure 1(b)). Flow cytometry analysis was performed to confirm that MSCs expressed cell surface antigens. In each MSC type, the positive markers CD73, CD90, and CD105 were expressed at 95% or higher, and the negative markers CD14, CD34, CD45, CD79a, and HLA-DR were expressed at less than 2% (Figure 1(c)). These results demonstrated that the human tissue-derived cells used in the present study had typical MSC characteristics (Supplementary Figure 1).

3.2. Commonly Secreted Proteins among the Three Types of MSCs. LC-MS/MS (n = 3 per tissue source) was performed to identify the proteins secreted by each MSC type under normal or inflammatory conditions (Figure 2(a)). We confirmed that the number of proteins secreted by each MSC type under normal or inflammatory conditions was 350 and 355 in BM-MSCs, 272 and 234 in AT-MSCs, and 136 and 138 in SM-MSCs, respectively (detailed information in Supplementary Table 1). Comparison of common proteins identified 105 proteins under normal conditions and 90 proteins under inflammatory conditions to be commonly secreted by BM-, AT-, and SM-MSCs (all-MSCs; Figure 2(b) and Table 1).

3.3. GO Analysis. GO analysis was performed to confirm the functional differences between commonly secreted proteins by all-MSCs under normal and inflammatory conditions. As a result, in MF, the percentage of “protein binding” of the secreted proteins under both conditions was confirmed to be 88% or more, while the percentages of other categories were 35% or less (Figure 3(a)). In CC, the percentage of “extracellular” of the secreted proteins under both conditions was confirmed to be 73% or more, and that of the other categories was 50% or less (Figure 3(b)). In BP, the percentage of secreted proteins under both conditions including “regulation of biological process,” “response to stimulus,” “metabolic process,” and “cell organization and biogenesis” was confirmed to be approximately 52% or more, with the percentages of these categories being higher under inflammatory conditions than under normal conditions. The percentages of other categories were confirmed to be 40% or less (Figure 3(c)).

3.4. KEGG Pathway Analysis. KEGG pathway analysis was conducted to identify signalling pathways related to proteins commonly secreted by all-MSCs under normal and inflammatory conditions. We confirmed that the pathway most closely related to proteins secreted under both conditions was “signal transduction.” The main pathways were similar under both conditions, but the levels of the secreted proteins were higher under inflammatory conditions than under normal conditions (Figure 4).

3.5. Changes in PPI Networks Depending on Environmental Differences. PPI network analysis was performed using the STRING database to confirm how the interaction networks of commonly secreted proteins by all-MSCs were altered because of environmental differences. Thus, excluding unconnected nodes, the PPI network under normal condition was composed of 102 nodes and 368 edges. The PPI network under inflammatory condition was composed of 92 nodes and 309 edges (interaction score > 0.7), confirming three main clusters under each condition. Under normal conditions, “extracellular matrix (ECM) organization” constituted 13 proteins, primarily including the collagen family and “regulation of cell differentiation” constituted 29 proteins, including proteins such as insulin-like growth factor-binding protein 3/4/7, gelsolin, and stanniocalcin-2. In addition, “cellular component organization” constituted 5 proteins, including vinculin (VCL) (Figure 5(a)). Under the inflammatory condition, “ECM organization” constituted 10 proteins, including those from the collagen family, and “protein metabolic process” constituted 5 proteins, including matrix metalloproteinase (MMP). Moreover, “response to stimulus” constituted 12 proteins including C-X-C motif chemokine 2/3/6/8 and annexin A1 (Figure 5(b)).

3.6. Identification of Proteins with Tissue Regeneration Potential. We identified proteins related to tissue regeneration among the commonly secreted proteins by all-MSCs under normal and inflammatory conditions. The categories affecting tissue regeneration were classified as anti-inflammation, anti-apoptosis, ECM-cell interactions, homeostasis,
inhibition of MMPs, and regeneration of chondrocytes. Proteins related to tissue regeneration were found to be secreted under all or specific environments. Except for the category of ECM-cell interactions, the number of secreted proteins was larger under inflammatory conditions than under normal conditions. We identified that proteins such as TSG-6 and thrombospondin 1 (TSP-1) are secreted only under inflammatory conditions (Table 2).

3.7. Validation of Commonly Secreted Proteins by Three Types of MSCs under the Two Environmental Conditions. ELISA was performed to validate the proteins presented in Table 2. We confirmed that TSP-2 was secreted under both environmental conditions, whereas TSG-6 was secreted under inflammatory conditions. The expression level of TSP-2, confirmed to be secreted under the two conditions, was higher under the normal condition than under the inflammatory condition. However, the concentration of TSP-2 in the CM was not significantly different between the normal and inflammatory conditions (Figure 6(a)). The expression level of TSG-6 was surprisingly 15 times higher under the inflammatory condition than under the normal condition (Figure 6(b)). The control was serum-free medium, and no protein was detected in it.

4. Discussion

We investigated proteins predicted to exhibit cartilage regeneration potential by confirming the differences and characteristics of proteins commonly secreted by human BM-, AT-, and SM-MSCs, depending on environmental differences. Thus far, cartilage repair studies using MSCs derived from various tissues, including BM, AT, and SM, have reported these cells to exhibit similar cartilage regenerative properties despite differences in paracrine factors secreted by each MSC type [46–48]. Based on these results, we hypothesised that there are paracrine factors commonly secreted by MSCs derived from tissues of different origins. Therefore, MSCs were isolated from each tissue; the cultured cells were confirmed to possess MSC characteristics defined
by the International Society for Cellular Therapy. The paracrine factors secreted by each MSC type were then identified using LC-MS/MS analysis. The identified proteins were validated by ELISA, finding differences in the secreted proteins depending on the tissue origin of MSCs and the presence or absence of inflammatory factors (Supplementary Table 1).

GO analysis was performed to confirm the properties of commonly secreted proteins by all-MSCs under normal or inflammatory conditions. We found that the largest proportion of MF was “protein binding,” suggesting that direct regulation of PPIs may be a major regulatory process carried out by proteins secreted by MSCs [49]. BP included “regulation of biological process,” “response to stimulus,” “metabolic process,” and “cell organization and biogenesis,” indicating that processes functionally relevant to commonly secreted proteins by all-MSCs play an important role in tissue repair [23, 49].

In addition, KEGG pathway analysis was conducted to confirm the biological function of paracrine factors commonly secreted by all-MSCs, and several pathways related to regeneration were verified. Environmental information processing included several pathways such as signal transduction, and the subcategories of signal transduction were identified in 11 pathways (data not shown). Among these pathways, the TGF-β signalling pathway is known to be essential for tissue regeneration by activating Smad signalling.

**Table 2: The identification of proteins related to tissue regeneration.**

| Anti-inflammation | Anti-apoptosis | ECM-cell interactions | Homeostasis | Inhibition of MMPs | Regeneration of chondrocytes |
|-------------------|----------------|-----------------------|-------------|-------------------|-----------------------------|
| ANXA1 [59, 60]    | LTF [70]       | BGN [73]              | TSG-6 [6, 65, 66] | ANXA1 [59, 60]    | TSP-2 [81, 82] |
| TSP-1 [62, 64, 69]| TMSB4X [68]   | COL1A1 [74]           | INHBA [75]  | TSP-2 [81, 82]    | LTF [70] |
| TSG-6 [6, 65, 66]| LIF [71]       | IGFBP3 [75]           | TSP-1 [62, 64, 69] | VIM [78]          | MIF [83] |
| NID1 [67]         | CALR [72]      | DCN [73]              | TNF [80]    | ANXA1 [59, 60]    | TSP-2 [81, 82] |
| TMSB4X [68]       |                | LUM [73]              | TNF [80]    |                   | LTF [70] |
|                   |                | FBN1 [63]             | TNF [80]    |                   | MIF [83] |
|                   |                | FN1 [63, 75]          | TNF [80]    |                   |                 |
|                   |                | VCL [76]              | TNF [80]    |                   |                 |
|                   |                | TSP-1 [63]            | TNF [80]    |                   |                 |
|                   |                | TSP-2 [63]            | TNF [80]    |                   |                 |
|                   |                | TFP12 [77]            | TNF [80]    |                   |                 |

**Normal condition**

| NID1 [67]         | LTF [70]       | CTGF [86]             | TSP-2 [81, 82] | CYR61 [88]       | TSP-2 [81, 82] |
| SERPINE1 [79]     | ACTG1 [74]     | TGFBI [87]            | SOD1 [89]    | SPOCK1 [90]      | LTF [70] |
| A2M [84, 85]      | VTN [74]       | VTN [74]              | A2M [84]     | IGFBP3 [75]      | MIF [83] |
|                   |                | COL1A1 [74]           | IGFBP3 [75]  | TSP-2 [81, 82]   |                 |
|                   |                | IGFBP3 [75]           | DCN [73]     | TSP-2 [81, 82]   |                 |
|                   |                | DCN [73]              | LUM [73]     | TSP-2 [81, 82]   |                 |
|                   |                | LUM [73]              | FN1 [63]     | TSP-2 [81, 82]   |                 |
|                   |                | LUM [73]              | VCL [76]     | TSP-2 [81, 82]   |                 |
|                   |                | FBN1 [63]             | VCL [76]     | TSP-2 [81, 82]   |                 |
|                   |                | FN1 [63, 75]          | VCL [76]     | TSP-2 [81, 82]   |                 |
|                   |                | VCL [76]              | VCL [76]     | TSP-2 [81, 82]   |                 |
|                   |                | TSP-2 [63]            | VCL [76]     | TSP-2 [81, 82]   |                 |

**Figure 6:** Validation experiments of key protein expression under various knee OA conditions using ELISA: (a) human TSP-2; (b) human TSG-6. ***P < 0.0001. ns: not significant (n = 9, three donors for each MSC type); N-CM: normal condition-conditioned medium; I-CM: inflammatory condition-conditioned medium.
to regulate collagen and aggrecan expression [50, 51]. In addition, this pathway has been reported to maintain homeostasis and inhibit the degradation of articular chondrocytes by modulating proinflammatory cytokines, eventually repairing or regenerating damaged cartilage tissue [52]. Taken together, each MSC type secreted proteins related to the TGF-β signalling pathway, and these proteins may contribute to the maintenance and regeneration of damaged cartilage.

We confirmed that the processes related to repair and regeneration were more under inflammatory conditions by GO and KEGG pathway analyses. The mechanism underlying this phenomenon was studied using PPIs. We found that ECM organization was a process observed under both environmental conditions. ECM stores and appropriately supplies factors necessary for cell growth and differentiation and supports spontaneous regeneration mechanisms to recover damaged tissues, displaying potential as a therapeutic avenue [53]. Meanwhile, as expected, a “cluster of responses to stimuli” was formed under inflammatory conditions. According to several studies reporting the association of paracrine factors secreted by MSCs under inflammatory conditions, the secretion and therapeutic effects of MSCs may be enhanced by inflammatory stimuli [54–56]. In addition, secretion of paracrine factors is reportedly increased in MSCs stimulated with inflammatory factors [41, 57]. These findings indicate that paracrine factors contribute to the regeneration of damaged tissue under any condition. Furthermore, it was confirmed that paracrine factors secreted under inflammatory conditions are closely related to the repair and regeneration of cartilage, and several studies have reported them to regulate endogenous cellular responses and mediate cartilage regeneration in damaged tissue [6, 58]. Thus, we concluded that paracrine factors released under inflammatory conditions facilitate the recovery and regeneration of damaged cartilage.

Table 2 [6, 59–91] shows the proteins predicted to be involved in the regeneration of damaged tissue. It was confirmed that ECM-cell interactions included collagen α-1(I) chain, decorin, biglycan, lumican, VCL, fibronectin 1, TSP-1, and TSP-2. These proteins are primarily known to constitute cartilage ECM and provide structural support to cells and tissues. Furthermore, they modulate cellular signals that can affect tissue organization, cellular proliferation, matrix adhesion, growth factors, and cytokine responses. They also reportedly protect the surface of collagen type I and II fibres from degradation [63, 92–94]. The articular cartilage, a specialised form of hyaline cartilage, is avascular and has a poor capacity for self-repair [95]. Therefore, ECM-cell interactions are considered essential for cartilage regeneration, and the proteins identified herein are similar to those reported in other studies [94, 96].

Knee OA development has been observed to increase proinflammatory cytokines such as IL-1β and TNF-α in the joint cavity, which can trigger chondrocyte apoptosis [97, 98]. In addition, lactoferrin (LTF) was reported to inhibit IL-1β-mediated chondrocyte apoptosis by regulating the activity of cyclic AMP-responsive element-binding protein through protein kinase B signalling. LTF also promoted cartilage regeneration by increasing the expression of collagen type II in a rat OA model. Thus, LTF secreted by human MSCs can be anticipated to have anti-apoptotic and regenerative effects on chondrocytes in damaged cartilage. In a study of TSP-2, Jeong et al. [82] showed that intraarticular injection of human umbilical cord blood-MSCs into an osteochondral defect rat model exerts a regenerative effect on damaged cartilage through paracrine factors, of which TSP-2 was the major paracrine player. These findings demonstrate the therapeutic potential of TSP-2-mediated paracrine action of human MSCs, which can regenerate cartilage in knee OA treatment.

Furthermore, inflammation is accompanied by the breakdown of the ECM, the main component of articular cartilage [99]. The synovial fluid of knee OA patients exhibits a high level of proinflammatory cytokines as well as different types of MMPs [100]. Synovitis is mostly associated with gradual progressive damage to cartilage and severe pain. The pain in knee OA may be caused by the activation of nociceptive pathways by nerve growth factor, which in turn may occur due to inflammation. Therefore, inflammation is a major therapeutic target to relieve OA pain, and alleviation of inflammation and suppression of MMPs may be key strategies for knee OA treatment [101]. Interestingly, in our study, we found that TSG-6 and TSP-1 proteins were secreted only under inflammatory conditions. These proteins attenuate proinflammatory cytokines and MMPs by modulating the nuclear factor κB pathway and enhance the production of collagen type II [65, 102–104]. TSG-6 and TSP-1 have anti-inflammatory and tissue-protective properties and can enable the regeneration of damaged cartilage.

We confirmed the level of expression depending on the environment for some of the abovementioned proteins. TSP-2 was identified in both conditions, and it was confirmed that its expression level was similar between the two conditions. This suggests that TSP-2 is a paracrine factor that is secreted by MSCs under various conditions and plays an essential role in cartilage regeneration. TSG-6, which was only secreted under the inflammatory condition, showed a dramatically higher expression level under the inflammatory condition than under the normal condition, supporting the result that TSG-6 is induced by stimulation by IL-1 and TNF-α [105].

The limitation of this study is that the identified proteins could not be confirmed to regenerate damaged cartilage through actual studies. However, it was meaningful to check the protein list for paracrine factors secreted by MSCs under normal culture conditions compared to those secreted under inflammatory conditions. Moreover, according to recent studies, the paracrine factors identified in this study have an effect on cartilage regeneration [62–66]. Thus, commonly secreted paracrine factors by each MSC type provide valuable information for understanding the potential of damaged knee OA regeneration.

5. Conclusions

We identified proteins commonly secreted by human BM-, AT-, and SM-MSCs under normal and inflammatory
conditions and consequently found paracrine factors that are predicted to be closely related to cartilage regeneration under inflammatory conditions. Therefore, our study sheds light on paracrine factors as potential therapeutic options for knee OA.

Data Availability
The experimental data analysed in this study are included in this article. The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest
The authors declare that there is no conflict of interest regarding the publication of this paper.

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Supplementary Materials
Supplementary 1. Supplementary Figure 1: the characterizations of bone marrow (BM), adipose tissue (AT), and synovial membrane (SM) mesenchymal stem cells (MSCs). (a) Phase-contrast microscopy images of MSCs (scale bar = 100 μm). (b) Differentiation potential of MSCs. Adipogenic differentiation (Oil Red O, top), osteogenic differentiation (Alizarin Red S, middle), and chondrogenic differentiation (Alcian Blue, bottom). (c) The immunophenotyping of MSCs using flow cytometry. Positive (CD73, CD90, and CD105) and negative (CD14, CD34, CD45, CD79a, and human leukocyte antigen-DR (HLA-DR)) markers. The histogram is shown with an overlay isotype control.

Supplementary 2. Supplementary Table 1: detailed information on proteins secreted by BM-, AT-, and SM-MSC under normal and inflammatory conditions, respectively.

References
[1] M. F. Pittenger, D. E. Discher, B. M. Péault, D. G. Phinney, J. M. Hare, and A. I. Caplan, “Mesenchymal stem cell perspective: cell biology to clinical progress,” *NPJ Regenerative Medicine*, vol. 4, no. 1, p. 22, 2019.
[2] M. B. Murphy, K. Moncivais, and A. I. Caplan, “Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine,” *Experimental & Molecular Medicine*, vol. 45, no. 11, article e54, 2013.
[3] C. de Bari, F. Dell’Accio, P. Tylzanowski, and F. P. Luyten, “Multipotent mesenchymal stem cells from adult human synovial membrane,” *Arthritis & Rheumatology*, vol. 44, no. 8, pp. 1928–1942, 2001.
[4] M. F. Pittenger, A. M. Mackay, S. C. Beck et al., “Multilineage potential of adult human mesenchymal stem cells,” *Science*, vol. 284, no. 5411, pp. 143–147, 1999.
[5] V. A. Ferraris, “How do cells talk to each other?: paracrine factors secreted by mesenchymal cells,” *The Journal of Thoracic and Cardiovascular Surgery*, vol. 151, no. 3, pp. 849–851, 2016.
[6] P. Mancuso, S. Raman, A. Glynn, F. Barry, and J. M. Murphy, “Mesenchymal stem cell therapy for osteoarthritis: the critical role of the cell secretome,” *Frontiers in Bioengineering and Biotechnology*, vol. 7, p. 9, 2019.
[7] A. I. Caplan and J. E. Dennis, “Mesenchymal stem cells as trophic mediators,” *Journal of Cellular Biochemistry*, vol. 98, no. 5, pp. 1076–1084, 2006.
[8] M. Varela-Eirin, J. Loureiro, E. Fonseca et al., “Cartilage regeneration and ageing: targeting cellular plasticity in osteoarthritis,” *Aging Research Reviews*, vol. 42, pp. 56–71, 2018.
[9] K. Shah, A. G. Zhao, and H. Sumer, “New approaches to treat osteoarthritis with mesenchymal stem cells,” *Stem Cells International*, vol. 2018, Article ID 5373294, 9 pages, 2018.
[10] S. Castorina, C. Guglielmino, P. Castrogiovanni et al., “Clinical evidence of traditional vs fast track recovery methodologies after total arthroplasty for osteoarthritic knee treatment. A retrospective observational study,” *Muscles, Ligaments and Tendons Journal*, vol. 7, no. 3, pp. 504–513, 2017.
[11] W. Chen, Y. Sun, X. Gu et al., “Conditioned medium of mesenchymal stem cells delays osteoarthritis progression in a rat model by protecting subchondral bone, maintaining matrix homeostasis, and enhancing autophagy,” *Journal of Tissue Engineering and Regenerative Medicine*, vol. 13, no. 9, pp. 1618–1628, 2019.
[12] C. De Bari and A. J. Roelofs, “Stem cell-based therapeutic strategies for cartilage defects and osteoarthritis,” *Current Opinion in Pharmacology*, vol. 40, pp. 74–80, 2018.
[13] F. Barry and M. Murphy, “Mesenchymal stem cells in joint disease and repair,” *Nature Reviews Rheumatology*, vol. 9, no. 10, pp. 584–594, 2013.
[14] J. Matas, M. Orrego, D. Amenabar et al., “Umbilical cord-derived mesenchymal stromal cells (MSCs) for knee osteoarthritis: repeated MSC dosing is superior to a single MSC dose and to hyaluronic acid in a controlled randomized phase I/II trial,” *Stem Cells Translational Medicine*, vol. 8, no. 3, pp. 215–224, 2019.
[15] N. Saulnier, E. Viguer, E. Perrier-Groult et al., “Intra-articular administration of xenogeneic neonatal mesenchymal stromal cells early after meniscal injury down-regulates metalloproteinase gene expression in synovium and prevents cartilage degradation in a rabbit model of osteoarthritis,” *Osteoarthritis and Cartilage*, vol. 23, no. 1, pp. 122–133, 2015.
[16] L. Xu, Y. Wu, Z. Xiong, Y. Zhou, Z. Ye, and W. S. Tan, “Osteoarthritis with mesenchymal stem cells early after meniscal injury down-regulates metalloproteinase gene expression in synovium and prevents cartilage degradation in a rabbit model of osteoarthritis,” *Osteoarthritis and Cartilage*, vol. 23, no. 7, pp. 849–858, 2015.
[17] J. Denkovskij, E. Bagdonas, I. Kusleviciute et al., “Paracrine potential of the human adipose tissue-derived stem cells to modulate balance between matrix metalloproteinases and their inhibitors in the osteoarthritic cartilage in vitro,” *Stem Cells International*, vol. 2017, Article ID 9542702, 13 pages, 2017.
[18] V. Trachana, E. Mourmoura, I. Papathanasiou, and A. Tsezou, “Understanding the role of chondrocytes in osteoarthritis: utilizing proteomics,” *Expert Review of Proteomics*, vol. 16, no. 3, pp. 201–213, 2019.
[19] S. Khatab, G. van Osch, N. Kops et al., “Mesenchymal stem cell secretome reduces pain and prevents cartilage damage...
in a murine osteoarthritis model,” European Cells & Materials, vol. 36, pp. 218–230, 2018.

[20] A. T. Wang, Y. Feng, H. H. Jia, M. Zhao, and H. Yu, “Application of mesenchymal stem cell therapy for the treatment of osteoarthritis of the knee: a concise review,” World Journal of Stem Cells, vol. 11, no. 4, pp. 222–235, 2019.

[21] M. Lo Monaco, G. Mercks, J. Ratajczak et al., “Stem cells for cartilage repair: preclinical studies and insights in translational animal models and outcome measures,” Stem Cells International, vol. 2018, Article ID 9079538, 22 pages, 2018.

[22] Y. Nakashima, S. Nahar, C. Miyagi-Shiohira et al., “A liquid chromatography with tandem mass spectrometry-based proteomic analysis of primary cultured cells and subcultured cells using mouse adipose-derived mesenchymal stem cells,” Stem Cells International, vol. 2019, Article ID 7274057, 97 pages, 2019.

[23] J. Ma, J. Wu, L. Han et al., “Comparative analysis of mesenchymal stem cells derived from anniotic membrane, umbilical cord, and chorionic plate under serum-free condition,” Stem Cell Research & Therapy, vol. 10, no. 1, p. 19, 2019.

[24] A. O. Pires, B. Mendes-Pinheiro, F. G. Teixeira et al., “Unveiling the differences of secretome of human bone marrow mesenchymal stem cells, adipose tissue-derived stem cells, and human umbilical cord perivascular cells: a proteomic analysis,” Stem Cells and Development, vol. 25, no. 14, pp. 1073–1083, 2016.

[25] A. al Madhoun, S. K. Marafie, D. Haddad et al., “Comparative proteomic analysis identifies EphA2 as a specific cell surface marker for Wharton’s jelly-derived mesenchymal stem cells,” International Journal of Molecular Sciences, vol. 21, no. 17, p. 6437, 2020.

[26] D. Kehl, M. Generali, A. Mallone et al., “Protemic analysis of human mesenchymal stromal cell secretomes: a systematic comparison of the angiogenic potential,” NPJ Regenerative Medicine, vol. 4, no. 1, p. 8, 2019.

[27] M. Hassan Famian, S. Montazer Saheb, and A. Montaseri, “Conditioned medium of Wharton’s jelly derived stem cells can enhance the cartilage specific genes expression by chondrocytes in monolayer and mass culture systems,” Advanced Pharmaceutical Bulletin, vol. 7, no. 1, pp. 123–130, 2017.

[28] S. Nahar, Y. Nakashima, C. Miyagi-Shiohira et al., “A comparison of proteins expressed between human and mouse adipose-derived mesenchymal stem cells by a proteome analysis through liquid chromatography with tandem mass spectrometry,” International Journal of Molecular Sciences, vol. 19, no. 11, p. 3497, 2018.

[29] L. Balducci and G. Alessandri, “Isolation, expansion, and immortalization of human adipose-derived mesenchymal stromal cells from biopsies and liposuction specimens,” Methods in Molecular Biology, vol. 1416, pp. 259–274, 2016.

[30] H. Koga, T. Muneta, Y. J. Ju et al., “Synovial stem cells are regionally specified according to local microenvironments after implantation for cartilage regeneration,” Stem Cells, vol. 25, no. 3, pp. 689–696, 2007.

[31] J. Fan, R. R. Varshney, L. Ren, D. Cai, and D. A. Wang, “Synovium-derived mesenchymal stem cells: a new cell source for musculoskeletal regeneration,” Tissue Engineering Part B Reviews, vol. 15, no. 1, pp. 75–86, 2009.

[32] H. Yoshimura, T. Muneta, A. Nimura, A. Yokoyama, H. Koga, and I. Sekiya, “Comparison of rat mesenchymal stem cells derived from bone marrow, synovium, periosteum, adipose tissue, and muscle,” Cell and Tissue Research, vol. 327, no. 3, pp. 449–462, 2007.

[33] Y. Sakaguchi, I. Sekiya, K. Yagishita, and T. Muneta, “Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source,” Arthritis & Rheumatology, vol. 52, no. 8, pp. 2521–2529, 2005.

[34] M. Dominici, K. le Blanc, I. Mueller et al., “Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement,” Cytotherapy, vol. 8, no. 4, pp. 315–317, 2006.

[35] L. Janderová, M. McNeil, A. N. Murrell, R. L. Mynatt, and S. R. Smith, “Human mesenchymal stem cells as an in vitro model for human adipogenesis,” Obesity Research, vol. 11, no. 1, pp. 65–74, 2003.

[36] F. Langenbach and J. Handschel, “Effects of dexamethasone, ascorbic acid and beta-glycerophosphate on the osteogenic differentiation of stem cells in vitro,” Stem Cell Research & Therapy, vol. 4, no. 5, p. 117, 2013.

[37] T. Kurth, E. Hedbom, N. Shintani et al., “Chondrogenic potential of human synovial mesenchymal stem cells in alginate,” Osteoarthritis and Cartilage, vol. 15, no. 10, pp. 1178–1189, 2007.

[38] E. Matsumura, K. Tsuji, K. Komori, H. Koga, I. Sekiya, and T. Muneta, “Pretreatment with IL-1ß enhances proliferation and chondrogenic potential of synovium-derived mesenchymal stem cells,” Cytotherapy, vol. 19, no. 2, pp. 181–193, 2017.

[39] J. Platas, M. I. Guillén, M. D. del Caz, F. Gomar, V. Mirabet, and M. J. Alcaraz, “Conditioned Media from Adipose-Tissue-Derived Mesenchymal Stem Cells Downregulate Degradative Mediators Induced by Interleukin-1ß in Osteoarthritic Chondrocytes,” Mediators of Inflammation, vol. 2013, Article ID 357014, 10 pages, 2013.

[40] J. R. Ferreira, G. Q. Teixeira, S. G. Santos, M. A. Barbosa, G. Almeida-Porada, and R. M. Gonçalves, “Mesenchymal stromal cell secretome: influencing therapeutic potential by cellular pre-conditioning,” Frontiers in Immunology, vol. 9, p. 2837, 2018.

[41] C. Li, G. Li, M. Liu, T. Zhou, and H. Zhou, “Paracrine effect of inflammatory cytokine-activated bone marrow mesenchymal stem cells and its role in osteoblast function,” Journal of Bioscience and Bioengineering, vol. 121, no. 2, pp. 213–219, 2016.

[42] S. Y. An, Y. J. Jang, H. J. Lim et al., “Milk fat globule-EGF factor 8, secreted by mesenchymal stem cells, protects against liver fibrosis in mice,” Gastroenterology, vol. 152, no. 5, pp. 1174–1186, 2017.

[43] J. R. Wiśniewski, A. Zougman, N. Nagaraj, and M. Mann, “Universal sample preparation method for proteome analysis,” Nature Methods, vol. 6, no. 5, pp. 359–362, 2009.

[44] R. Konietzny, R. Fischer, N. Ternte et al., “Detection of BK virus in urine from renal transplant subjects by mass spectrometry,” Clinical Proteomics, vol. 9, no. 1, p. 4, 2012.

[45] S. Um, H. Y. Kim, J. H. Lee, I. S. Song, and B. M. Seo, “TSG-6 secreted by mesenchymal stem cells suppresses immune reactions influenced by BMP-2 through p38 and MEK mitogen-activated protein kinase pathway,” Cell and Tissue Research, vol. 368, no. 3, pp. 551–561, 2017.

[46] C. H. Jo, Y. G. Lee, W. H. Shin et al., “Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a proof-of-concept clinical trial,” Stem Cells, vol. 32, no. 5, pp. 1254–1266, 2014.
H. Chen, X. H. Min, Q. Y. Wang et al., “Arthroscopic transplantation of synovial stem cells improves clinical outcomes in knees with cartilage defects,” *Clinical Orthopaedics and Related Research*, vol. 473, no. 7, pp. 2316–2326, 2015.

X. Xing, S. Han, G. Cheng, Y. Ni, Z. Li, and Z. Li, “Proteomic analysis of exosomes from adipose-derived mesenchymal stem cells: a novel therapeutic strategy for tissue injury,” *BioMed Research International*, vol. 2020, Article ID 6094562, 10 pages, 2020.

Y. Zhu, H. Tao, C. Jin et al., “Transforming growth factor-β1 induces type II collagen and aggrecan expression via activation of extracellular signal-regulated kinase 1/2 and Smad2/3 signaling pathways,” *Molecular Medicine Reports*, vol. 12, no. 4, pp. 5573–5579, 2015.

W. Wang, D. Rigueur, and K. M. Lyons, “TGFβ signaling in cartilage development and maintenance,” *Birth Defects Research Part C Embryo Today*, vol. 102, no. 1, pp. 37–51, 2014.

J. A. Roman-Blas, D. G. Stokes, and S. A. Jimenez, “Modulation of TGF-β signaling by proinflammatory cytokines in articular chondrocytes,” *Osteoarthritis and Cartilage*, vol. 15, no. 12, pp. 1367–1377, 2007.

C. Somaiah, A. Kumar, D. Mawrie et al., “Collagen promotes higher adhesion, survival and proliferation of mesenchymal stem cells,” *PloS One*, vol. 10, no. 12, p. e0145068, 2015.

L. Liu, Y. R. He, S. J. Liu et al., “Enhanced Effect of IL-1β-Activated Adipose-Derived MSCs (ADMSCs) on Repair of Intestinal Ischemia-Reperfusion Injury via COX-2-PGE2 Signaling,” *Stem Cells International*, vol. 2020, Article ID 2803747, 18 pages, 2020.

E. Maffioli, S. Nonnis, R. Angioni et al., “Proteomic analysis of the secretome of human bone marrow-derived mesenchymal stem cells primed by pro-inflammatory cytokines,” *Journal of Proteomics*, vol. 166, pp. 115–126, 2017.

H. Chen, X. H. Min, Q. Y. Wang et al., “Pre-activation of mesenchymal stem cells with TNF-α, IL-1β and nitric oxide enhances its paracrine effects on radiation-induced intestinal injury,” *Scientific Reports*, vol. 5, no. 1, p. 8718, 2015.

G. Ren, L. Zhang, X. Zhao et al., “Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide,” *Cell Stem Cell*, vol. 2, no. 2, pp. 141–150, 2008.

W. Chen, J. Cai, Y. Sun, J. Chen, and S. Chen, “Research progress in treatment of knee osteoarthritis by paracrine effect of stem cells,” *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi = Chinese Journal of Reparative & Reconstructive Surgery*, vol. 33, no. 11, pp. 1446–1451, 2019.

M. A. Sugimoto, J. P. Vago, M. M. Teixeira, and L. P. Sousa, “Annexin A1 and the resolution of inflammation: modulation of neutrophil recruitment, apoptosis, and clearance,” *Journal of Immunology Research*, vol. 2016, Article ID 8239258, 13 pages, 2016.

N. J. Goulding, J. Dixey, E. F. Morand et al., “Differential distribution of annexins-I, II, IV, and VI in synovium,” *Annals of the Rheumatic Diseases*, vol. 54, no. 10, pp. 841–845, 1995.

H. Yang, R. Zhao, H. Chen, P. Jia, L. Bao, and H. Tang, “Bornyl acetate has an anti-inflammatory effect in human chondrocytes via induction of IL-11,” *IUBMB Life*, vol. 66, no. 12, pp. 854–859, 2014.

M. Maumus, C. Manferdini, K. Toupet et al., “Thrombospondin-1 partly mediates the cartilage protective effect of adipose-derived mesenchymal stem cells in osteoarthritis,” *Frontiers in Immunology*, vol. 8, p. 1638, 2017.

J. Halper and M. Kjaer, “Basic components of connective tissues and extracellular matrix: elastin, fibrillin, fibulins, fibrinogen, fibronectin, laminin, tenascins and thrombospondins,” *Advances in Experimental Medicine and Biology*, vol. 802, pp. 31–47, 2014.

B. Jeremiesse, C. Matta, C. R. Fellows et al., “Alterations in the chondrocyte surfaceome in response to pro-inflammatory cytokines,” *BMC Molecular and Cell Biology*, vol. 21, no. 1, p. 47, 2020.

A. J. Day and C. M. Milner, “TSG-6: a multifunctional protein with anti-inflammatory and tissue-protective properties,” *Matrix Biology*, vol. 78-79, pp. 60–83, 2019.

K. A. Jha, M. Pentecost, R. Lenin et al., “TSG-6 in conditioned media from adipose mesenchymal stem cells protects against visual deficits in mild traumatic brain injury model through neurovascular modulation,” *Stem Cell Research & Therapy*, vol. 10, no. 1, p. 318, 2019.

J. Kruegel, B. Sadowski, and N. Miosge, “Nidogen-1 and nidogen-2 in healthy human cartilage and in late-stage osteoarthritis cartilage,” *Arthritis & Rheumatology*, vol. 58, no. 5, pp. 1422–1432, 2008.

W. S. Yang, S. Kang, J. Sung, and H. K. Kleinman, “Thymosin β4: potential to treat epidermolysis bullosa and other severe dermal injuries,” *European Journal of Dermatology*, vol. 29, no. 5, pp. 459–467, 2019.

M. T. Sweetwyne and J. E. Murphy-Ullrich, “Thrombospondin1 in tissue repair and fibrosis: TGF-β-dependent and independent mechanisms,” *Matrix Biology*, vol. 31, no. 3, pp. 178–186, 2012.

N. Brandl, A. Zemann, I. Kauep et al., “Signal transduction and metabolism in chondrocytes is modulated by lactoferrin,” *Osteoarthritis and Cartilage*, vol. 18, no. 1, pp. 117–125, 2010.

M. Salehnia, M. Fayazi, and S. Ehsani, “Leukemia inhibitory factor increases the proliferation of human endometrial stromal cells and expression of genes related to pluripotency,” *International Journal of Reproductive Biomedicine*, vol. 15, no. 4, pp. 209–216, 2017.

L. van Duyn Graham, M. T. Sweetwyne, M. A. Pallero, and J. E. Murphy-Ullrich, “Intracellular calreticulin regulates multiple steps in fibrillar collagen expression, trafficking, and processing into the extracellular matrix∗,” *The Journal of Biological Chemistry*, vol. 285, no. 10, pp. 7076–7078, 2010.

J. Melrose, E. S. Fuller, P. J. Roughley et al., “Fragmentation of decorin, biglycan, lumican and keratan is elevated in degenerate human meniscus, knee and hip articular cartilages compared with age-matched macroscopically normal and control tissues,” *Arthritis Research & Therapy*, vol. 10, no. 4, p. R79, 2008.

H. Luo, L. Yao, Y. Zhang, and R. Li, “Liquid chromatography-mass spectrometry-based quantitative proteomics analysis reveals chondroprotective effects of astragaloside IV in interleukin-1β-induced SW1353 chondrocyte-like cells,” *Biomedicine & Pharmacotherapy*, vol. 91, pp. 796–802, 2017.
Y. Fujihara, A. Hikita, T. Takato, and K. Hoshi, “Vinculin in cell-cell and cell-matrix adhesions,” Cellular and Molecular Life Sciences, vol. 74, no. 16, pp. 2999–3009, 2017.

M. Lino, D. C. Foster, and W. Kisiel, “Quantification and characterization of human endothelial cell-derived tissue factor pathway inhibitor-2,” Arteriosclerosis, Thrombosis, and Vascular Biology, vol. 18, no. 1, pp. 40–46, 1998.

B. E. Bobick, R. S. Tuan, and F. H. Chen, “The intermediate filament vimentin regulates chondrogenesis of adult human bone marrow-derived multipotent progenitor cells,” Journal of Cellular Biochemistry, vol. 109, no. 1, pp. 265–276, 2010.

K. Masuko, M. Murata, N. Suematsu et al., “A suppressive effect of prostaglandin E2 on the expression of SERPINE1 plasminogen activator inhibitor-1 in human articular chondrocytes: an in vitro pilot study,” Open access Rheumatology : Research and Reviews, vol. 1, pp. 9–15, 2009.

S. M. Riester, Y. Lin, W. Wang et al., “RNA sequencing identifies gene regulatory networks controlling extracellular matrix synthesis in intervertebral disk tissues,” Journal of Orthopaedic Research, vol. 36, no. 5, pp. 1356–1369, 2018.

K. Shin, Y. Cha, Y. H. Ban et al., “Anti-osteoarthritis effect of a combination treatment with human adipose tissue-derived mesenchymal stem cells and thrombospondin 2 in rabbits,” World Journal of Stem Cells, vol. 11, no. 12, pp. 1115–1129, 2019.

S. Y. Jeong, D. H. Kim, J. Ha et al., “Thrombospondin-2 secreted by human umbilical cord blood-derived mesenchymal stem cells promotes chondrogenic differentiation,” Stem Cells, vol. 31, no. 10, pp. 2136–2148, 2013.

Y. Fujihara, A. Hikita, T. Takato, and K. Hoshi, “Roles of macrophage migration inhibitory factor in cartilage tissue engineering,” Journal of Cellular Physiology, vol. 233, no. 2, pp. 1490–1499, 2018.

J. M. Cuellar, V. G. Cuellar, and G. J. Scuderi, “α2-Macroglobulin: autologous protease inhibition technology,” Physical Medicine and Rehabilitation Clinics of North America, vol. 27, no. 4, pp. 909–918, 2016.

Y. Luan, L. Kong, D. R. Howell et al., “Inhibition of ADAMTS-7 and ADAMTS-12 degradation of cartilage oligomeric matrix protein by alpha-2-macroglobulin,” Osteoarthritis and Cartilage, vol. 16, no. 11, pp. 1413–1420, 2008.

X. Xing, Z. Li, Z. Yu, G. Cheng, D. Li, and Z. Li, “Effects of connective tissue growth factor (CTGF/CCN2) on condylar chondrocyte proliferation, migration, maturation, differentiation and signalling pathway,” Biochemical and Biophysical Research Communications, vol. 495, no. 1, pp. 1447–1453, 2018.

B. Reinboth, J. Thomas, E. Hanssen, and M. A. Gibson, “βig-h3 interacts directly with biglycan and decorin, promotes collagen VI aggregation, and participates in ternary complexes with these macromolecules,” Journal of Biological Chemistry, vol. 281, no. 12, pp. 7816–7824, 2006.

M. Chijiwa, S. Mochizuki, T. Kimura et al., “CCN1 (Cyr61) is overexpressed in human osteoarthritic cartilage and inhibits ADAMTS-4 (aggrecanase 1) activity,” Arthritis & Rheumatology, vol. 67, no. 6, pp. 1557–1567, 2015.

M. Koike, H. Nojiri, H. Kanazawa et al., “Superoxide dismutase activity is significantly lower in end-stage osteoarthritic cartilage than non-osteoarthritic cartilage,” PLoS One, vol. 13, no. 9, p. e0203944, 2018.

H. J. Hauser, R. Decking, and R. E. Brenner, “Testican-1, an inhibitor of pro-MMP-2 activation, is expressed in cartilage,” Osteoarthritis and Cartilage, vol. 12, no. 11, pp. 870–877, 2004.

A. Santoro, J. Conde, M. Scotece et al., “SERPINE2 inhibits IL-1α-Induced MMP-13 expression in human chondrocytes: involvement of ERK/NF-κB/AP-1 pathways,” PLoS One, vol. 10, no. 8, article e0135979, 2015.

G. X. Ni, Z. Li, and Y. Z. Zhou, “The role of small leucine-rich proteoglycans in osteoarthritis pathogenesis,” Osteoarthritis and Cartilage, vol. 22, no. 7, pp. 896–903, 2014.

S. A. Flowers, A. Zieba, J. Örnros et al., “Lubricin binds cartilage proteins, cartilage oligomeric matrix protein, fibronectin and collagen II at the cartilage surface,” Scientific Reports, vol. 7, no. 1, article 13149, 2017.

H. Ragelle, A. Naba, B. L. Larson et al., “Comprehensive proteomic characterization of stem cell-derived extracellular matrices,” Biomaterials, vol. 128, pp. 147–159, 2017.

A. J. Sophia Fox, A. Bedi, and S. A. Rodeo, “The basic science of articular cartilage: structure, composition, and function,” Sports Health, vol. 1, no. 6, pp. 461–468, 2009.

A. Harvey, T. Y. Yen, I. Azizman, C. Tate, and C. Case, “Proteomic analysis of the extracellular matrix produced by mesenchymal stromal cells: implications for cell therapy mechanism,” PLoS One, vol. 8, no. 11, article e79283, 2013.

A. Mathiessen and P. G. Conaghan, “Synovitis in osteoarthritis: current understanding with therapeutic implications,” Arthritis Research & Therapy, vol. 19, no. 1, p. 18, 2017.

Y. Hu, Z. Gui, Y. Zhou, L. Xia, K. Lin, and Y. Xu, “Quercetin alleviates rat osteoarthritis by inhibiting inflammation and apoptosis of chondrocytes, modulating synovial macrophages polarization to M2 macrophages,” Free Radical Biology and Medicine, vol. 145, pp. 146–160, 2019.

C. H. Evans, D. C. Mears, and J. L. McKnight, “A preliminary ferrographic survey of the wear particles in human synovial fluid,” Arthritis & Rheumatology, vol. 24, no. 7, pp. 912–918, 1981.

J. C. Fernandez, J. Martel-Pelletier, and J. P. Pelletier, “The role of cytokines in osteoarthritis pathophysiology,” Biochemistry, vol. 39, no. 1-2, pp. 237–246, 2002.

P. G. Conaghan, A. D. Cook, J. A. Hamilton, and P. P. Tak, “Therapeutic options for targeting inflammatory osteoarthritis pain,” Nature Reviews Rheumatology, vol. 15, no. 6, pp. 355–363, 2019.

W. Xu, S. Li, F. Yu et al., “Role of thrombospondin-1 and nuclear Factor-xB signaling pathways in antiangiogenesis of infantile hemangioma,” Plastic and Reconstructive Surgery, vol. 142, no. 3, pp. 310e–321e, 2018.

S. Rosini, N. Pugh, A. M. Bonna, D. J. S. Hulmes, R. W. Farn-dale, and J. C. Adams, “Thrombospondin-1 promotes matrix homeostasis by interacting with collagen and lysyl oxidase precursors and collagen cross-linking sites,” Science Signaling, vol. 11, no. 532, article eaar2566, 2018.
[104] H. Choi, R. H. Lee, N. Bazhanov, J. Y. Oh, and D. J. Prockop, “Anti-inflammatory protein TSG-6 secreted by activated MSCs attenuates zymosan-induced mouse peritonitis by decreasing TLR2/NF-κB signaling in resident macrophages,” *Blood*, vol. 118, no. 2, pp. 330–338, 2011.

[105] T. H. Lee, L. Klampfer, T. B. Shows, and J. Vilcek, “Transcriptional regulation of TSG6, a tumor necrosis factor- and interleukin-1-inducible primary response gene coding for a secreted hyaluronan-binding protein,” *Journal of Biological Chemistry*, vol. 268, no. 9, pp. 6154–6160, 1993.