Conditioned Medium of IGF1-Induced Human Wharton's Jelly Mesenchymal Stem Cells Effects on Sox9 Gene Expression and Level of COL2 and IL1β in Osteoarthritic Chondrocytes

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Abstract. Wharton's jelly mesenchymal stem cells (WJMSCs) hold promises for treating osteoarthritis (OA) due to their great capacity for regeneration and their accessibility. However, obstacles like poor cell survival and differentiation prevent them from being used in transplants. Thus, this study examined WJMSCs' Conditioned Medium (CM) (WJMSCs-CM) for OA treatment. In order to determine how well IL1β-induced chondrocyte cells (IL1β-CHON002) heal OA-damaged cells, they were treated with both IGF1-induced and uninduced WJMSCs-CM. Key parameters measured were IL1β, Sox9 and COL2. The STRING database was explored to evaluate the proteins contained in WJMSCs-CM and to see their interactions and CM potential in OA exposure. The STRING database indicates WJMSCs-CM to possess the ability to manage OA because of the growth factors they contain, which raise the expression of cartilage marker genes. The results indicated that 15% WJMSCs-CM with 150 ng/mL IGF1 had the highest Sox9 expression, significant reduction in pro-inflammatory mediators, and an increase in COL2 levels. Additionally, 14 days of treatment resulted in better outcomes compared to 7 days. CM from WJMSCs treated with IGF1 with a concentration of 15% exhibited the highest level for each parameter. Both durations showed difference across parameters, highlighting the potential for OA therapy.

Keywords: *collagen 2; IGF-1; IL-1β; sox9; WJMSCs.*

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1 Introduction

Musculoskeletal diseases, including joint calcification or osteoarthritis (OA) and rheumatoid arthritis (RA), continue to increase because life expectancy and the elderly population continue to increase [1]. Pathologic alterations in OA joints involve the deterioration of articular cartilage, enlargement of the subchondral bone, formation of osteophytes, degeneration of ligaments, joint capsule hypertrophy, and synovial inflammation, all of which can disrupt the homeostasis associated with OA [2,3]. The goal of OA treatment is typically not to rebuild articular cartilage. Clinical intervention is typically based on the primary symptoms and concentrates on lowering inflammation-related pain by utilizing non-steroidal anti-inflammatory medication (NSAIDs) or even complete joint replacement [3]. Long-term consumption of NSAIDs can cause side effects in the form of kidney disorders, indigestion, and cardiovascular disease [4][5].

The most popular option for treating OA is chondrocytes, a component of cartilage [6]. However, chondrocyte therapy has drawbacks, such as the need to perform surgery twice, which may cause further damage and degeneration of the cartilage. In addition, the differentiation of chondrocytes during culture can also produce fibrocartilage with low regeneration ability. Because multipotent stem cells may develop into a variety of cell types, like chondrocytes, they provide greater promise for tissue engineering and cartilage repair. They have demonstrated immunomodulatory properties, making them strong agents for regenerative therapies [7]. Autologous Mesenchymal stem cells (MSCs) can be extracted from the patient in order to treat OA. Allogeneic MSCs, on the other hand, must come from two or more different donors [8]. As a result, OA patients require a different strategy to rebuild cartilage.

Stem cells (SCs) are a part of the regenerative strategies for tissue regeneration that can be multiplied in large quantities. The preparation and method of administration of SCs for therapy is relatively easy. MSCs are perfect for cartilage repair therapy because of their capacity to differentiate into chondrocytes. MSCs can also influence the microenvironment by suppressing the immune system and reducing inflammation. Hence, MSCs reduce the progressive nature of OA [9]. In addition, MSCs can be used for allogeneic application therapy. MSCs can shield cartilages from harm and stimulate regeneration of the remaining progenitor cells by secreting a variety of bioactive soluble substances [10].

In particular, Wharton's Jelly Mesenchymal Stem Cells (WJMSCs) hold promises for the therapeutic approach of OA due to their extraordinary capacity for regeneration and accessibility. When OA is still in its early stages, WJMSCs may help with knee function and pain response [11]. However, stem cell transplants face challenges such limited cell differentiation and survival. Conditioned medium

(CM) derived from stem cell cultivation can be used to solve these issues. Secreted components known as the secretome, microvesicles, or exosomes are present in CM. Numerous studies have demonstrated that the released factor, without the stem cell itself, may lead to tissue restoration in a range of disorders involving tissue/organ injury [12]. Because CM is easier to work with, there are several benefits to exploiting the secretome it carries as opposed to stem cells. Furthermore, the receiver and donor do not have to match in order to prevent rejection issues when using CM. As a result, the production of CM as a drug for regenerative medicine is a promising prospect [13]. Moreover, CM does not comprise live materials, which provides another advantage, as there is no risk of cell death during culture or delivery. Additionally, therapies involving CM reduce both cost and time, as there is no need to wait for cells to mature during the process. This also allows for the medium to be collected multiple times from cells that have been seeded only once [14]. With the objective of creating an in vitro model of OA, IL1β, an inflammatory cytokine, was used to induce chondrocytes (CHON002), which led to the expression of several pro-inflammatory proteins and a decrease in the expression of markers that promote chondrogenesis [15]. The CM of human WJMCs (hWJMSCs-CM) contains a variety of important proteins, including growth factors, cytokines, and angiogenic factors. The present investigation assessed the prospects of MSCs from Wharton's jelly as an OA treatment by looking at the expression of the Sox9 gene and the levels of IL1β and COL2 in CM from IL1β-CHON002, treated with CM from untreated hWJMSCs (hWJMSCs-CM), and hWJMSCs induced by insuline-like growth factor (IGF1-hWJMSCs-CM).

2 Materials and Method

2.1 STRING Database Analysis

Analysis of protein interactions was performed using the STRING database to search for the association of proteins found in WJMSCs-CM with proteins

Tabel 1 Cytokines, growth factor, and angiogenic factors found in WJMSCs-CM.

Cytokines	Growth Factors	Angiogenic Factors
IFNα	HGF	ANG
IL1RN	PDGFα	ANGPT1
IL6	TGFβ1	COL15A1
IL7	TGFβ2	FGF1
IL8	TGFβ3	PLGF
IL12	BMP	THBS2

IFNα = Interferon α, HGF = Hepatic Growth Factor, ANG = Angiotensin, IL1RN = Interleukin 1 Receptor Antagonist, PDGFα = Platelet-derived Growth Factor Alpha, ANGPT1 = Angiopoietin 1, IL = Interleukin, TGF = Transforming Growth Factor, COL = Collagen, FGF1 = Fibroblast Growth

Factor 1, PLGF = Placental Growth Factor, BMP = Bone Morphogenetic Protein, THBS2 = Thrombospondin 2.

related to OA. The proteins in Tables 1 and 2 were entered into the STRING database search page (https://string-db.org/). The selected organism was *Homo sapiens*. The data obtained was analyzed and modified, resulting in relevant data regarding the interactions of proteins contained in WJMSCs-CM with proteins related to OA. The interaction data was then visualized using the Inkscape software.

Tabel 2 Cartilage protein markers associated with OA.

Cartilage Markers	Hypertrophic Differentiation Marker (OA)
ACAN	GREM1
COL2A1	COL1A1
SOX9	RUNX2
	COL10A1
	IHH
	AXIN2
	ADAMTS
	MMP1

ACAN = Aggrecan, COL = Collagen, GREM = Gremlin, RUNX2 = Runt-related transcription factor 2, IHH = Indian Hedgehog Homolog, SOX9 = SRY-type High-mobility Group Box-9, AXIN2 = axis inhibition protein 2, ADAMTS = A Disintegrin and Metalloproteinase with Thrombospondin motifs, MMP1 = Matrix Metalloproteinase 1.

2.2 Culture of CHON002 and hWJMSCs

Aretha Medika Utama provided human WJMSCs for Passage 4 (P4). The cells were described using multipotent differentiation and cell surface phenotype. The cell culture methods used on the WJMSCs were as described by Widowati [11,14]. The human long bone cartilage (CHON002 ATCC CRL-2847TM) was provided by Aretha Medika Utama, Bandung, Indonesia. The hWJMSCs were cultivated and maintained at a density of 1 x 10^6/well in a 6-well plate using the minimal essential medium-α (MEM-α) (Gibco, 12561056) supplemented with 10% (v/v) FBS (Gibco, 10082147), 1% antibiotic and antimycotic (Gibco, 15240062), and 1% geneticin (Gibco, 10151027). The cells were incubated at 37 ºC in a humidified environment with 5% CO_2. Following seeding in Dulbecco's Modified Eagle Media (DMEM, Gibco 11995065), the CHON002 cells, which had a density of 1 x 10^6/well in a 6-well plate, were incubated for two to three days. After adding 5 or 10 ng/mL of recombinant IL1β (Biolegend, 579404) to the media, the cells were stimulated for five days [16][17].

2.3 Induction of hWJMSCs with IGF1 and CM Collection

Briefly, 3 x 10^5 cells per well in a six-well plate were subcultured into a T25 flask (SPL 70075) and incubated at 37 °C with 5% CO_2 until 70% confluence was reached in three days. Recombinant IGF1 was inserted to the medium at doses of

0 and 150 ng/mL. The hWJMSCs-CM were gathered during the induction and replacement of the medium. The resulting CM was kept at -80 °C in 50 mL tubes. The WJMSCs were induced for one week, using recombinant IGF1 (Biolegend, 1500102) [17].

2.4 Induction of CHON002 with IL1β

Briefly, 5 x 10^5 CHON002 were subcultured in a T25 flask (SPL 70075), and the flask was incubated at 37 °C with 5% CO_2 until 70% confluence was achieved. Recombinant IL1β (Biolegend 511604) was introduced to the medium at levels of 0 and 10 ng/mL after five days. The following procedure was employed to supply IL1β-CHON002 with hWJMSCs-CM and IGF1-hWJMSCs-CM: 1) normal CHON002, 2) IL1β-CHON002, 3) IL1β-CHON002 + hWJMSC-CM 15%, 4) IL1β-CHON002 + hWJMSC-CM 30%, 5) IL1β-CHON002 + IGF1-hWJMSCs-CM 15%, 6) IL1β-CHON002 + IGF1-hWJMSC-CM 30%. Every two days, the medium for hWJMSCs and IGF1-hWJMSCs was switched. CM treatment was administered for one and two weeks to IL1β-CHON002 as an OA model [16][17][18].

2.5 Post-Treatment CHON002 RNA Isolation

After receiving CM treatment for one or two weeks, CHON002 cells were collected using trypsin-EDTA for three minutes in an incubator set at 37 °C with 5% CO_2. After adding the entire medium and transferring it to a 15 mL tube, the trypsin was inactivated by centrifuging it for five minutes at 1,600 g. Without the supernatant, the pellet was resuspended in 350 µL of 70% ethanol and 350 µL of lysis solution. RNA isolation was performed employing the Aurum-RNA Solution Kit (BioRad, 7326820) in accordance with the manufacturer's protocol. The RNA was preserved at -80 °C until the subsequent steps [17][18].

2.6 Synthesis of cDNA CHON002 Post-Treatment

Following the manufacturer's instructions, cDNA synthesis was carried out employing the iScript cDNA synthesis kit (BioRad 1708890). To initiate the reaction, a master mix or sample including 5x iScript 4 µL, RT iScript 1 µL, 50 ng RNA sample, and nuclease-free water was created. The mixture was subjected into incubation in gradient PCR using the subsequent procedures: five minutes at 25 °C for priming, thirty minutes at 42 °C for reversing, five minutes at 85 °C for RT inactivation, and five minutes at 4 °C for holding. The resulting synthesized cDNA was maintained at -80 °C [17,18].

2.7 qPCR Analysis of Sox9 Gene Expression

For the qPCR reaction, the SsoFast Eva Green Supermix kit was utilized in accordance with the manufacturer's instructions (BioRad 172-5200). Three reactions/samples were created using the kit, which included three 10 µL evagreen, three 2 µL reverse and forward primers, three 5 µL nuclease-free water, and three 1 µL cDNA samples. The master mix was relocated to a piko real plate and then incubated in a ThermoScience qPCR machine using the following steps: 5 minutes of pre-denaturation at 95 °C, 1 minute of denaturation at 95 °C, 1 minute of elongation at 72 °C, 40 seconds of annealing at the temperature listed in Table 3, 5 minutes of post-elongation at 72 °C, melt curve at 55-90 °C, and holding at 4 °C [19][17]. The sequence of the designed primer is shown in Table 3.

Tabel 3 Primer design used for the analysis of Sox9 and GAPDH expressions.

Gene	Primer	Sequence	Annealing Temperature	References
Sox9	Reverse	5'-TTCGGTTATTTTTAGGATCATCTCG-3'	53 °C	NM_000346.4
	Forward	5'-CACACAGCTCACTCGACCTTG-3'	53 °C	
GAPDH	Reverse	5'-TGGCAGGTTTTTCTAGACGG-3'	53 °C	NM_001357943.2
	Forward	5'-GGGCTGCTTTTAACTCTGGT-3'	53 °C	

2.8 ELISA Assay of IL1β and COL2 Levels in IL1β-CHON002 as OA Model

The procedure for the ELISA method to test multiple protein expressions of IL1β and COL2 in CM of IL1β-CHON002 was carried out based on the issued protocol (Elabscience E-EL-H0149 /H0774). In short, standards or samples were placed into each well and incubated at 37 °C for 90 minutes. After discarding the liquid in the well, 100 µL of the Biotinylated Detection Antibody kit was added. The plates were kept at 37 °C for 60 minutes to incubate. After removing the liquid from the plate, it was cleaned three times using washing buffer. After 100 µL of HRP Conjugate was added to the well, the mixture was subjected to incubation at 37 °C for 30 minutes. After removing the liquid from the plate, it was cleaned five times with washing buffer. The dish was tapped on absorbent paper to dry it. The reagent substrate (90 µL) was applied to each well and put into incubation at 37 °C for 15 minutes. Subsequently, 50 µL of stop solution was added. Color changes that occurred were observed and immediately scanned (450 nm) [16][18].

2.9 Statistical Analysis

Three replications of the procedures were carried out. The SPSS software (version 20.0) was operated to do the statistical analysis. The data was displayed using the mean ± standard deviation. The Bonferroni Post-hoc Test was applied to evaluate

if there were any significant differences among the groups following the analysis of variance (One Way ANOVA). The Friedman test was used to examine the effects of IGF1-hWJMSCs-CM 15% and 30% therapy on each parameter over the course of one and two weeks in order to ascertain the significance of treatment duration.

3 Result

3.1 Search Results Using the STRING Database

Growth factor interaction with cartilage markers and hypertrophic differentiation markers can be seen in Figure 1. CM contains 2 cytokines and 4 growth factors that affect the cartilage markers. Meanwhile, hypertrophic differentiation markers affected by cytokines and 3 growth factors contained in CM.

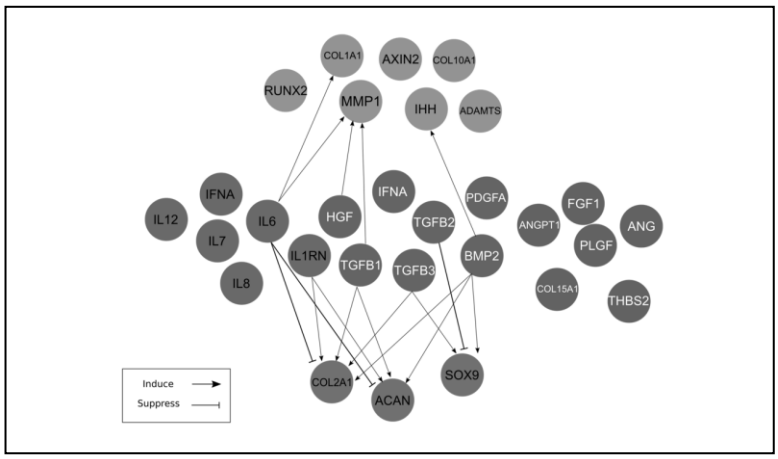

Figure 1 Network of protein interactions possessed by the proteins found in hWJMSCs-CM with proteins related to OA.

3.2 Results of the Expression of Sox9 Analysis

The addition of IL1β into CHON002 without treatment hWJMSCs-CM resulted in lower expression of chondrogenesis marker Sox9 compared to normal CHON002. This study proved that the addition IGF1-hWJMSCs-CM, which is rich in proteins, could increase chondrogenic differentiation. The results of the expression analysis of the Sox9 gene is shown in Figure 2. Based on this result, the IGF1-hWJMSC-CM 15% treatment was the most effective in increasing Sox9 gene expression ($P < 0.05$). Overall, all hWJMSCs-CM treatments exhibited a promotion of Sox9 gene expression.

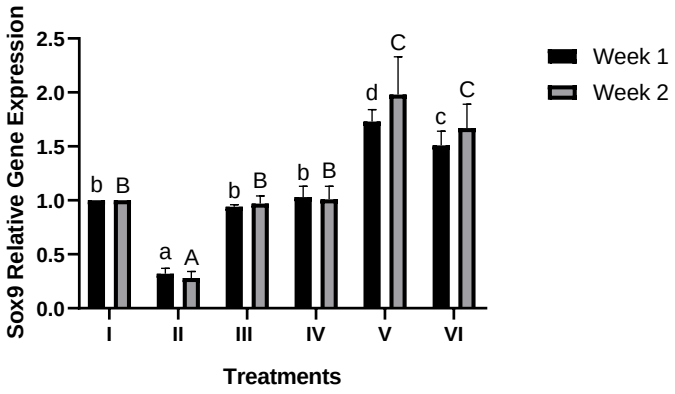

Figure 2 Sox9 gene expression. I: normal CHON002, II: IL1β-CHON002, III: IL1β-CHON002 + hWJMSCs-CM 15%, IV: IL1β-CHON002 + hWJMSCs-CM 30%, V: IL1β-CHON002 + IGF1-hWJMSCs-CM 15%, VI: IL1β-CHON002 + IGF1-hWJMSCs-CM 30%. (* The level of Sox9 expression was measured in triplicate. The letters 'a', 'b', 'c', and 'd' for week 1 and the letters 'A', 'B', and 'C' for week 2 indicate significant differences between the treatments in each week.

In addition, the result of Sox9 gene expression was increased from 1 week to 2 weeks of treatment, meaning that time plays an important role in recovery or cell regeneration in OA therapy with hWJMSCs-CM through increased expression of Sox9 transcription factors.

3.3 Results of IL1β and COL2 Level Analysis

In Figure 3, it can be seen that CHON002 induced with IL1β had the highest IL1β levels and showed an inflammatory condition, while administration of IGF1-hWJMSCs-CM 15% suggested a notable decrease in IL1β ($P < 0.05$) in comparison. Additionally, all treatments displayed a significant reduction in comparison with the untreated OA cell model (IL1β-CHON002).

The level of COL2 in each hWJMSCs-CM treatment can be seen on Figure 4. Increased levels of COL2 on CHON002 induced by IL1β occurred after administration of IGF1-hWJMSC-CM 30%, which approximated COL2 levels in normal cells (CHON002). In addition, treatment for 2 weeks also resulted in increasing levels of COL2 with treatment of IGF1-hWJMSCs-CM 30% and 15% resulted in the highest COL2 level.

The IGF1-hWJMSCs-CM 15% treatment resulted in the highest level of each parameter. The Friedman test was used to analyze the impact of the IGF1-

hWJMSCs-CM 15% treatment between the 1-week and 2-week durations on each parameter. The results indicated no significant differences, suggesting that the treatment had a similar effect across both time points.

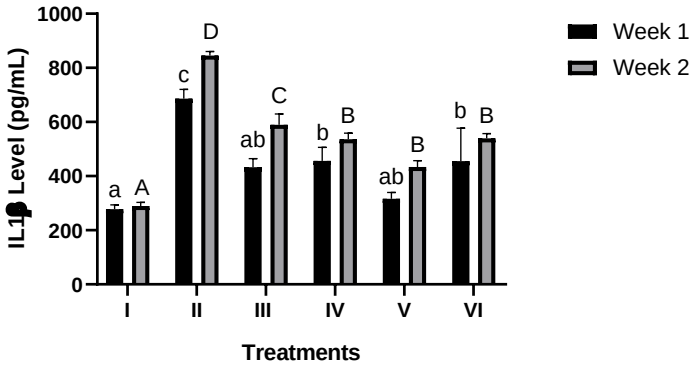

Figure 3 Effect of hWJMSCs-CM, IGF1-hWJMSCs-CM toward IL1β level on OA cells model. I: normal CHON002, II: IL1β-CHON002, III: IL1β-CHON002 + hWJMSC-CM 15%, IV: IL1β-CHON0020 + hWJMSC-CM 30%, V: IL1β-CHON002 + IGF1-hWJMSCs-CM 15%, VI: IL1β-CHON002 + IGF1-hWJMSC-CM 30%. The level of IL1β expression was measured in triplicate. The data were analyzed as mean ± standard deviation. The letters 'a', 'ab', 'b', 'c' for week 1 and the letters 'A', 'B', 'C', and 'D' for week 2 indicate significant differences between the treatments in each week.

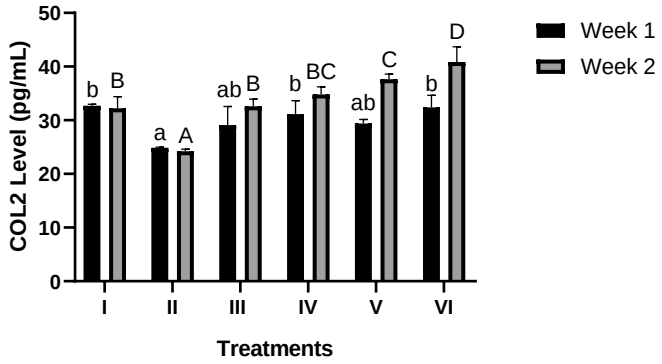

Figure 4 Effect of hWJMSCs-CM, IGF1-hWJMSCs-CM toward COL2 level on OA cells model. I: normal CHON002, II: IL1β-CHON002, III: IL1β-CHON002 + hWJMSC-CM 15%, IV: IL1β-CHON002 + hWJMSC-CM 30%, V: IL1β-CHON002 + IGF1-hWJMSCs-CM 15%, VI: IL1β-CHON002 + IGF1-hWJMSC-CM 30%. The level of COL2 was measured in triplicate. The letters 'a', 'ab', and 'b' for week 1 and the letters 'A', 'B', 'BC', 'C', and 'D' for week 2 indicate significant differences between the treatments in each week.

4 Discussion

The slow shift from chondrogenic development to cartilage mineralization, referred to as chondrocyte hypertrophic differentiation, is described by a variety of markers. Every one of these indicators plays a distinct part in the mineralization of cartilage [16][19]. The STRING database did not reveal relevant interactions of angiogenic factors found in hWJMSCs-CM with proteins related to OA. Cytokines interact with cartilage markers and hypertrophic differentiation markers. Only anti-inflammatory cytokines induce cartilage markers, while pro-inflammatory cytokines suppress the expression of cartilage markers and induce hypertrophic differentiation markers. Interleukin-1 receptor antagonist (IL-1RNA) is needed by Matrilin-3 (MATN3) to promote Collagen, type II, alpha 1 (COL21A) and Aggrecan (ACAN) expression in human chondrocyte cells. Administration of siRNA IL1RNA can prevent the expression of COL21A and ACAN [20]. IL6 treatment can suppress some ACAN gene expression in chondrocyte articular cartilage [21]. IL6 inhibits transcription of COL2A1 in chondrocyte articular cartilage [22]. IL6 can increase COL1A1 expression in lung fibroblast-like cells and human fetal lung fibroblast [23]. IL6 contributes in increasing the expression of MMP1 in fibroblasts [24]. It is known that growth factors, especially the Transforming Growth Factor-Beta (TGFβ) protein family, induce protein expression of cartilage markers. The TGFβ1 induces COL2A1 and ACAN gene expression in monolayer chondrocytes and 3D-alginate cultures [25]. While TGFβ3 deficiency can lead to osteogenesis, TGFβ3 treatment increased the expression of COL2A1 and ACAN on bone marrow-derived mesenchymal stem cells (BMMSCs) [26]. BMP2 intensifies the expression of the COL2A1 gene and synthesis of ACAN and proteoglycan in healthy and damaged cartilages [27]. TGFβ2 increases Sox9 gene expression in alginate chondrocyte cultures [26]. Bone Morphogenetic Protein 2 (BMP2) increases Sox9 gene expression in cardiac endothelial cell cultures [28]. Growth factors also induce hypertrophic differentiation markers. TGFβ1 administration resulted in increased expression of Matrix Metalloproteinase 1 (MMP1) 1 in the host's osteoblast cells [29]. Hepatocyte Growth Factor (HGF) increases the expression of MMP1 through ERK signaling pathways in fibroblasts [30]. BMP2 can increase IHH gene expression in rabbit chondrocyte growth plates [31].

Most proteins found in hWJMSCs-CM affect the cartilage markers. Growth factors are considered to play a vital role in the healing potential of hWJMSCs-CM against OA, because some growth factors induce the expression of extracellular cartilage matrix genes. Based on the data obtained, the angiogenic factors found in hWJMSCs-CM do not affect cartilage markers or hypertrophic differentiation markers. However, it is important to know that angiogenesis plays an important role in inflammation, which can worsen the prognosis of OA [32]. Anti-inflammatory cytokines also have a big contribution in collagen expression, but

pro-inflammatory cytokines suppress cartilage markers while inducing hypertrophic differentiation markers. Future application of CM from hWJMSCs in the treatment of OA is possible. However, specific pre-treatments are necessary for hWJMSCs to reduce angiogenic factors and pro-inflammatory cytokines while enhancing the secretion of growth factors and anti-inflammatory cytokines.

Sox9 is a transcription factor that can control chondrogenic differentiation. This factor is also important in chondrogenic differentiation due to its role in regulating the transcription initiation of other genes related in chondrogenesis [33]. TGFβ2 increases Sox9 gene expression in alginate chondrocyte cultures [26]. BMP2 increases Sox9 gene expression in cardiac endothelial cell culture and co-administration of BMP binding endothelial regulator (BMPER) can prevent this [28]. The gene-expression profile of HGF-stimulated DU145 prostate cancer cells, characterized by increased Sox9 expression HGF, has been linked to several tissues as a paracrine regulator of organogenesis and repair [34]. One of the regulations is the proliferation and differentiation of cartilage in mouse c-met receptor in cells [35].

In primary lung fibroblast cultures, Platelet-derived Growth Factor (PDGFα) boosted Sox9 in addition to HGF through PDGFRα active signaling, which is necessary to sustain Sox9 [36]. In both chondrocytes and undifferentiated mesenchymal cells, fibroblast growth factors (FGFs) also promote the expression of the gene for the master chondrogenic factor Sox9. It is highly probable that the Mitogen-Activated Protein Kinase (MAPK) pathway mediates the regulation [37]. In differentiated human bone marrow stem cells (hBMSCs), IL8 significantly elevated the levels of Sox9 and COL2 [38]. In this study, hWJMSCs-CM treatments in all concentrations was able to upregulate Sox9 expression. This corresponds with a previous study that reported that overexpression of Sox9 led to the reduction of cartilage destruction in OA mice [39].

In general, inflammation occurs due to the induction of various internal mediators like cytokines and proteases, as well as internal cellular mechanisms that have an impact on increasing the production of inflammatory mediators. OA is a disease caused by aging chondrocytes in joints. In this disease, chondrocytes have a limited capacity to proliferate and have the capacity to secrete various mediators that induce inflammation that support elevation of oxidative stress [40]. The primary mediators of inflammation and increased matrix degradation by proteases such MMP and A Disintegrin and Metalloproteinase with Thrombospondin Motifs (ADAMTS) in the course of OA illness are Tumor Necrosis Factor (TNFα) and IL1β [41]. OA can be brought on by IL1β in a number of ways, including unbalanced cartilage repair processes, COX2, and inducible nitric oxide synthase (iNOS). Additionally, it can lead to the development of ROS and promote the

synthesis of inflammatory mediators such as prostaglandin E2 [42][43]. As a result, IL1β is utilized as a biomarker for the pathogenesis of OA.

The synthesis of IL1Ra in human liver (HepG2) cells cultured with increasing HGF levels is negatively correlated with the dosage of HGF [44]. Due to its capacity to counteract the NO production induced by IL1 and the catabolic effects of IL1 on articular cartilage both in vitro and in vivo, TGFβ has been suggested as a protective factor for articular cartilage [45]. Transcriptional regulation of rat sarcoma virus GTPase (rasGTPase) and ubiquitin/proteasome pathways are two unique mechanisms that most likely influence the effect of TGFβ and its relationship with IL1 [46].

COL2 is one of the predominant constituents of the cartilage tissue extracellular matrix. The reorganization of COL2 in damaged cartilage tissue indicates that the regeneration process is underway. Hence, COL2 is considered to be one of the important markers of chondrocytes that are specialized in building cartilage tissue [47]. Aggrecan and type II collagen, two essential components of the cartilage matrix, can be produced more readily when TGF is induced [48]. Additionally, BMP2 markedly increases the expression of type II collagen in chondrocytes grown in three-dimensional (3D) alginate [49]. Thrombospondin-2 (TSP2) has not been found to affect the collagen 2 level directly but can affect collagen fibrillogenesis without being an integral component of fibrils [50]. PDGFα/β and TGFβ1 was able to synergistically increase protein synthesis, collagen production, and proliferation capacity in enzymatically isolated smooth muscle cells (SMCs) from the aortic media of young swine. PDGFα/β alone had no influence. This data exhibits mutual interactions for controlling and regulating proliferation and collagen synthesis of SMCs [51].

In mesangial cells, expression of proteoglycans and COL2 is affected by overexpression of Sox9. Analysis using double immunofluorescence microscopic revealed that when MCs were transiently transfected with FLAG-SOX9 plasmid, Sox9 was primarily induced in the nucleus, followed by the expression of COL2 in the cytoplasm [52]. An experiment in human intestinal smooth muscle cells has shown that IL1β down-regulates collagen synthesis. IL1β is a well-established mitogen and causes a notable inhibition of collagen synthesis. It is most likely that the latter effect, which has been observed on both the secretion of newly generated procollagen into culture media and its accumulation in cells, is caused by the suppression of procollagen production [53]. This fact is also related to secretion of MMP13 by osteoarthritic chondrocytes in response to IL1β, leading to digestion of COL2 in cartilage [54]. Additionally, IL1β reduced the mRNA level and protein expression of Sox9 and COL2 in cultured intervertebral disc cells ($P < 0.05$) in a dose-responsive manner [55]. However, this study demonstrated that IGF1-

hWJMSCs-CM treatments could upregulate COL2 level. Figure 5 displays the proposed mechanism of this study.

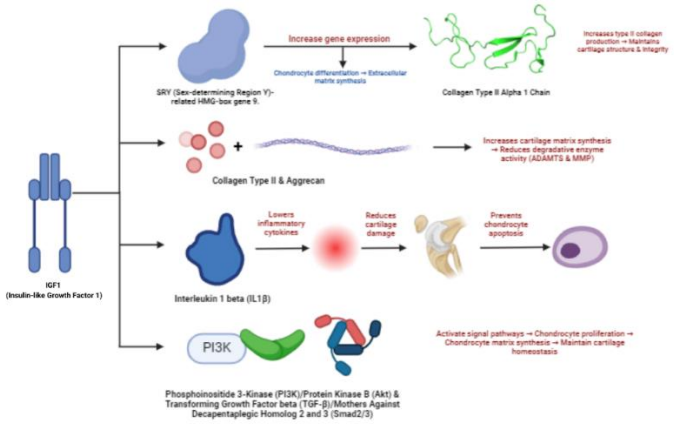

Figure 5 Proposed mechanism of IGF1 as an osteoarthritic therapy agent.

IGF1 ameliorates osteoarthritis through COL2 gene expression regulation, hence modulating cartilage structure and integrity. Moreover, it also lowers inflammatory cytokine, IL1β, contributing to chondrocyte apoptosis prevention.

5 Conclusion

This study demonstrated that the highest significant level of each parameter was obtained by CM from hWJMSCs cultured with 15% IGF1. As a result, the treatment is sufficiently successful to be used as OA therapy.

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