1. Interaction of viral oncogenic proteins with the Wnt signaling pathway

Sayyad Khamzadeh, Banafshe Hasani Ward, Hamed Esmaeil Kashgarian, Mohammad Almasi; Gholamreza Goudarzi

View Article: PDF 625.76 K

2. Spermatogenesis after transplantation of adipose tissue-derived mesenchymal stem cells in busulfan-induced azospermic hamster

Negar Karamaghaei, Amin Tamadon, Farhad Rahmani Far; Davood Mehrabani, Alireza Raayat Jahromi; Shahrin Zara; Zahra Khosobandeh, Imam Rezaei, Jahromi; Omid Kooch-Hoseini; Mehrad Dinapour

View Article: PDF 1.01 MB

3. The effect of vitamin C on mice hemolytic anemia induced by phenylhydrazine: an animal model study using histological changes in testis, pre-implantation embryo development and biochemical changes

Pages 668-677

Hojjat Anbari, Rasoul Shatoo; Maztaq Reza, Hassan Malekinejad, Gholamreza Najafi

View Article: PDF 1009.45 K

4. AGS cell line xenograft tumor as a suitable gastric adenocarcinoma model: growth kinetic characterization and immunohistochemistry analysis

Pages 673-681

Tahaeh Barati; Mahnaz Haddadi, Fatemeh Sadeghi; Samad Muhammadnejad; Abad Muhammadnejad, Ronak Heidarian, Motahhareh Abriamandnejad, Saied Amoupour

View Article: PDF 449.34 K

5. Protective effects of troxerutin on maternal high-fat diet-induced impairments of spatial memory and apelin in the male offspring

Pages 682-687

Rojaye Cita, Olou Mohaddes, Farida Mizzale Bavi, Fereshthe Farajadkar, Parvin Bayandor, Maryam Hosseindost; Kevan Mehri, Zeinbeh Zarvari Oskueyi, Shrin Babri

View Article: PDF 452.21 K

6. Human wild-type superoxide dismutase 1 gene delivery to rat bone marrow stromal cells: its importance and potential future trends

Pages 688-694

Mohsen Abedi, Seyed Alireza Mostah-Namin; Ali Noor-Zaideh; Taki Tiraith; Taher Taheri

View Article: PDF 720.95 K

7. Examination of the immunohistochemical localization and gene expression by RT-PCR of the oxytocin receptor in diabetic and non-diabetic mouse testes

Pages 695-700

Argit AYDOGAN; Seyft Ali BINGOL

View Article: PDF 1 MB

8. Lipoprotein lipase gene polymorphisms as risk factors for stroke: a computational and meta-analysis

Pages 701-706

Majid Negati, Mohammad Ali Atlasi; Mohammad Kariman, Hossein Nikzad; Aboozad Azami

View Article: PDF 952.74 K

9. Mild hypoxia and human bone marrow mesenchymal stem cells synergistically enhance expansion and homing capacity of human cord blood CD34+ stem cells

Pages 709-716

Fatemeh Mohammadzadeh; saied Abroun; Amir Alasti

View Article: PDF 1.09 MB
10. Gambogic acid inhibits LPS-induced macrophage pro-inflammatory cytokine production mainly through suppression of the p38 pathway
Pages 717-723
Ma Jianjun, Kangmao Huang, Yan Ma, Shuai Chen, Chao Liu, Zhi Shan, Xiangqian Fang
View Article  PDF 855.63 K

11. CX501 as an AMPA receptor positive modulator, improves the learning and memory in a rat model of Alzheimer's disease
Pages 724-730
Naaznin Mozafari, Ali Shamsizadeh, Iman Fatemi, Mohammad Allahavakoli, Amir Moghadam-Ahmadi, Elham Kaviani, Ayat Kaeidi
View Article  PDF 1009.14 K

12. The effects of ginsenoside Rb1 on fatty acid β-oxidation, mediated by AMPK, in the failing heart
Pages 731-737
Hong-fang Kong, Ai-Jie Hou, Ning-ning Liu, Bo-han Chen, Hua-shi Huang, Sheng-nan Dai
View Article  PDF 627.1 K

13. Design and synthesis of new esters of terpenoid alcohols as 16-lipoxygenase inhibitors
Pages 738-744
Hamid Sadeghian, Seyed Mohammad Seyedi, Zainab Jafari
View Article  PDF 591.94 K

14. Effects of insulin-like growth factor-induced Wharton jelly mesenchymal stem cells toward chondrogenesis in an osteoarthritis model
Pages 745-752
Hadiy Monowar, Eri Affah, Tjandraawati Mozafari, Ferry Sandra, Rizal Rizal, Aminta Amalia, Yukiko Armita, Indra Bachtar, Harry Must
View Article  PDF 815.2 K

15. Simultaneous administration of a fusion protein composed of pertussis toxin and filamentous hemagglutinin from Bordetella pertussis induces mucosal and systemic immune responses
Pages 753-769
Ali Torkashvand, Farzohn Bahrami, Minoo Adib, Sohela Aftary
View Article  PDF 960.96 K

16. Relationship of cell surface hydrophobicity with biofilm formation and growth rate: A study on Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli
Pages 760-769
Zulfiqar Ali Mirani, Amran Fatima, Shaista Urozj, Mubashar Aziz, Muhammad Khan, Tanveer Abbas
View Article  PDF 736.97 K
**Effects of insulin-like growth factor-induced Wharton jelly mesenchymal stem cells toward chondrogenesis in an osteoarthritis model**

Wahyu Widowati 1, Ervi Afifah 2, Tjandrawati Mozef 3, Ferry Sandra 4, Rizal Rizal 2, Annisa Amalia 2, Yukko Arinta 2, Indra Bachtiar 5, Harry Murti 5

1 Medical Research Center, Faculty of Medicine, Maranatha Christian University, Bandung 40164, West Java, Indonesia
2 Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung 40163, West Java, Indonesia
3 Research Center for Chemistry, Indonesian Institute of Sciences, Serpong, Indonesia
4 Department of Biochemistry and Molecular Biology, Faculty of Dentistry, Trisakti University, Jakarta, Indonesia
5 Stem Cell and Cancer Institute, Jakarta 13210, Indonesia

**ARTICLE INFO**

**Article type:** Original article

**Article history:** Received: Dec 5, 2017 Accepted: Apr 9, 2018

**Keywords:** Chondrogenesis, IGF-1, Mesenchymal stem cell, MMPs, Osteoarthritis

**ABSTRACT**

**Objectives:** This study aimed to determine the collagen type II (COL2) and SOX9 expression in interleukin growth factor (IGF-1)-induced Wharton’s jelly mesenchymal stem cells (WJMSCs) and the level of chondrogenic markers in co-culture IGF1-WJMSCs and IL1β-COLON002 as osteoarthritis (OA) cells model.

**Materials and Methods:** WJMSCs were induced with IGF1 (75, 150, and 300 ng/ml) to enhance their chondrogenesis capability. The gene expression of SOX9 and COL2 was evaluated with quantitative RT-PCR. Furthermore, IGF1-WJMSCs were co-cultured with IL1β-COLON002 cells in varied ratios (1:1, 2:1). Chondrogenic markers ADAMTS1, ADAMTS5, MMP3, MMP1, and RANKL were measured with ELISA.

**Results:** The IGF1-WJMSCs had an increased expression of COL2 and SOX9. ADAMTS1, ADAMTS5, MMP1, MMP3, and RANKL levels were decreased in the co-culture IGF1-WJMSCs and IL1β-COLON002.

**Conclusion:** The IGF1-induced WJMSCs were capable to enhance chondrogenesis, indicated by increased expression of SOX9 and COL2 and decreased expression of ADAMTS1, ADAMTS5, MMP3, MMP1, and RANKL. These findings can be further used in the osteoarthritis treatment.

**Introduction**

Osteoarthritis (OA) is one of the common joint disorders worldwide and the main cause of body-support disability. The characteristics of OA include phenotypic changes in the superficial layer cells of the articular cartilage (AC), chondrocyte hypertrophy and apoptosis, progressive formation of osteophyte, fibrillation of the AC, sclerosis of subchondral bone, and increased remodeling of the periarticular bone (1–3).

The chondrocyte phenotype is characterized by specific genes expressions, i.e., collagen type II and the transcription factor SOX9 (4, 5). COL2 is an essential abundant component in the cartilage extra cellular matrix (ECM). Therefore, the COL2 disruption and loss of other cartilage ECM components during degenerative joint diseases such as OA will lead to severe disability and aging-related health problems (6). This may be stimulated by complex pathogenic mechanisms that decreased matrix synthesis and upregulated pathways of tissue degradation (7).

Stem cells take a role in novel treatment strategies for both clinical situations. Johnson et al. discovered a drug candidate by screening the small molecules that induced mesenchymal stem cells (MSCs) chondrogenic differentiation (8). MSCs were studied for cartilage development (9), which may be helpful for the developmental programs in OA.

MSCs were collected from cartilage (bone marrow mesenchymal stem cells/BM-MSCs) and subsequently from adipose tissue, the placenta, dental pulp, umbilical cord, amnion (10), and Wharton’s Jelly (11). Adipose tissue-MSCs (AD-MSCs) have lower chondrogenesis ability than BM-MSCs. Induction of TGFβ2 and IGF1 in AD-MSCs may produce chondrocytes that are slightly inferior to BM-MSCs chondrocytes, as measured using chondrocytes markers including COL1A, COL2A1, and SOX-9 (12).

The insulin-like growth factor (IGF1) is an enhancer that is responsible for the rate of gene expression (13–16), and IGF-2 plays a role as a growth stimulant in a non-differentiated state and as a regulator for glucose in all stages of differentiation (17, 18). A study reported that plasmid-based upregulation of IGF-1 in rabbit chondrocytes encapsulated using alginate in vivo showed an ability to repair cartilage and accelerated subchondral bone reformation in osteochondral disorders (19). Thus, we aimed to observe IGF-1 induction effect on the gene expression of chondrogenic markers, SOX9 and COL2, in Wharton’s Jelly MSCs.

**Materials and Methods**

**Cell culture preparation**

The human Wharton’s Jelly mesenchymal stem cells (hWJMSCs) of passage 4 (P4) were collected from the...
Stem Cell and Cancer Institute (Jakarta, Indonesia). The cells had been characterized by the cell multipotent differentiation and surface phenotype (11, 20). Informed consent was obtained from the Institutional Ethics Committee at the Stem Cell and Cancer Institute (Jakarta, Indonesia).

The hWJMSCs at a density of 5 x 10^5/well were cultured in minimum essential medium-a (α-MEM) (Gibco, 12561056), supplemented with fetal bovine serum (20%) (FBS, Gibco, 10270106) and 1% antibiotic and anti-mycotic (Gibco, 15240062). They were incubated in a humidified atmosphere with 5% CO₂ at 37 °C for 24 hr. The medium was discarded and washed with Phosphate Buffered Saline (PBS).

hWJMSCs at density 1 x 10^6 cells/well was maintained in a complete medium. The cells were treated with IGF-1 (Biolegend, 590904) at concentrations of 75, 150, and 300 ng/ml, and incubated at 5% CO₂, 37 °C for 7 days, to obtain IGF1-induced WJMSCs cells for measuring SOX9 and COL2 gene expression (11, 20).

**Co-culture of IGF1-WJMSCs and IL1β-CHON002**

IGF1 75 ng/ml-induced WJMSCs, IGF1 120 ng/ml-induced WJMSCs (IGF1-WJMSC), IL1β 5 ng/ml-induced CHON002, and IL1β 10 ng/ml-induced CHON002 (IL1β-CHON002) cells were collected with PBS containing 0.15% (w/v) trypsin (2000 units/g) and 0.02% EDTA. The cell suspensions of IGF1-WJMSCs and IL1β-CHON002 were mixed at five ratios including WJMSCs alone, IGF1-WJMSCs and IL1β-CHON002 1:2, 1:1, 2:1, IGF1-WJMSCs alone, and IL1β-CHON002 alone, and CHON002 alone. The cells were stored at 37 °C in 5% CO₂ with medium changes three times per week. The co-culture was stained with alcan blue, after 2 weeks of incubation. Meanwhile, the conditioned medium (CM) was collected from the co-culture and evaluated by ELISA assay (21).

**Quantification of COL2 and SOX9**

RNA was extracted using the Aurum total RNA kit (Bio-Rad, 7326820) and checked its concentration. The RNA was used for cDNA synthesis using iScript cDNA synthesis kit (Bio-Rad, 1725200). Table 1 shows the primers used in this research.

| Primer | Forward | Reverse |
|--------|---------|---------|
| Beta actin | 5’-TCTGGCACCACTTCACATAAGT-3' | 5’-AGCACAGGCTGGATAGCAACG-3’ |
| SOX-9 | 5’-TTCGGTTATTTTAGATCGATCC-3’ | 5’-CACACAGCTCGACCTGGTG-3’ |
| COL-2 | 5’-GGCAATAGCAGTTACACTGACA-3’ | 5’-CGATAACACCTGGCCCCATT-3’ |

**Results**

**Levels of SOX9 and COL2**

SOX9 is present in differentiated chondrocytes and all chondrocyte progenitors during chondrogenesis. However, the expression is completely turned off in hypertrophic chondrocytes (22). SOX9 expression parallels that of the gene coding for COL2A1, a chondrocyte differentiation specific marker (23). SOX9 and COL2 were measured in WJMSCs induced by IGF1 (Figure 1).

Figure 1 shows that the expression of SOX9 and COL2 at IGF1 concentration 150 ng/ml was higher, with values of (1.15 ± 0.07) and (8.44 ± 0.44), respectively compared to IGF1 300 ng/ml, IGF1 75 ng/ml, and control. This indicates that IGF1-induced WJMSCs cells can improve chondrogenesis in controlling differentiation of chondrogenic cells, which can repair chondrocyte damage in OA. Co-culture of IL1β-induced CHON002 and IGF1-induced WJMSCs, we used IGF1 75 ng/ml and

**Figure 1.** Gene expression levels of SOX9 and COL2 on IGF1-induced WJMSCs

The data was presented as a histogram of mean±standard deviation. Different letters (a,b) are significant among various concentrations of IGF1 (0, 75, 150, and 300 ng/ml) toward level of SOX9 gene expression, different letters (a,b,c,d) are significant among concentrations of IGF toward COL2 gene expression based on Duncan’s post hoc test (P<0.05)
120 ng/ml for inducing WJMSCs.

**Level of ADAMTS1 and ADAMTS5**

ADAMTS1 is presented within cartilage and the synovium (24) and its expression is significantly upregulated in OA cartilage (25). ADAMTS1 and ADAMTS5 levels are shown in Figures 2 and 3, respectively.

In general, co-cultures of IGF1-induced WJMSCs and IL1β-induced CHON002 with ratio 1:2 showed lowest ADAMTS1 among treatments (Figure 2); co-culture of IGF 75 ng/ml-induced WJMSCs and IL1β 5 ng/ml-induced CHON002 (1:2) showed lowest ADAMTS1 level (3.15 ng/ml), and it was significantly different compared to the positive control, IL1β 5 ng/ml-induced CHON002, and IL1β 10 ng/ml-induced CHON002. However, there was no marked difference between IGF1-induced WJMSCs alone, in which ADAMTS1 levels in both treatments (IGF1 75 ng/ml, 120 ng/ml) were 11.44 and 10.31 ng/ml, respectively.

On the other hand, co-culture IGF1 120 ng/ml-induced WJMSCs and IL1β 10 ng/ml-induced CHON002 showed the lowest ADAMTS5 level (24.83 ng/ml), which was significantly different compared to positive control, IL1β 5 ng/ml-induced CHON002, and IL1β 10 ng/ml-induced CHON002.
ng/ml-induced CHON002 (39.48 and 45.58 ng/ml, respectively). The result was comparable to negative control, CHON002 alone without induction (25.36 ng/ml). Single culture and IGF1-induced WJMSCs alone (IGF1 75 ng/ml, 120 ng/ml) also presented the low levels of ADAMTS5 (25.89 and 26.95 ng/ml, respectively).

**Level of MMP1 and MMP3**

The levels of MMP1 and MMP3 in co-culture IGF1-WJMSCs and IL1β-CHON002 are shown in Figures 4 and 5, respectively.

The results of the present study show that both IGF1-WJMSCs itself and co-culture with IL1β-CHON002 significantly decreased the level of MMP1 (Figure 4) compared to positive control namely, IL1β 5 ng/ml-induced CHON002 and IL1β 10 ng/ml-induced CHON002 (7.19 and 8.73 ng/ml, respectively). These results were comparable to negative control, CHON002 alone without induction (6.49 ng/ml). Similar results were also found in MMP3 levels in which both IGF1-induced WJMSCs alone and co-culture significantly decreased the MMP3 level compared to IL1β-induced CHON002 (Figure 5).

**Level of RANKL**

The TNF molecules called RANKL (receptor activator of NFκB ligand) is the main regulator of bone remodeling
and development and activation of osteoclasts. The levels of RANKL can be seen in Figure 6.

The results obtained show that there was a significant difference in RANKL levels between the treatment of IGF1-WJMSCs and IL1β-CHON002 (Figure 6). Level of RANKL was reduced by both IGF1-WJMSCs alone and co-culture, and it was significant compared to the negative control, IL1β 5 ng/ml-induced CHON002, and IL1β 10 ng/ml-induced CHON002. Co-culture IGF1 75 ng/ml-induced WJMSCs and IL1β 5 ng/ml-induced CHON002 (2:1) showed the lowest RANKL levels among co-cultures.

Discussion

MSCs have been employed as one particular sector of tissue engineering that involves the repair replacement, or regeneration of cartilage tissue, due to their superior proliferative and differentiation capacities (26). It has been reported that WJMSCs may differentiate into chondrocytes, skeletal muscle cells, cardiac muscle cells, osteoblasts, adipocytes, ,β cells in the islets of Langerhans, or endothelial cells in vitro (27). Hence, these cells may be applied in the treatment of chronic degenerative disorders and prevent cartilage degradation in patients with OA through their trophic/regenerative potential.

In the present study, treating WJMSCs with IGF1 increased expression of SOX9 (Figure 1). Many cartilage matrix genes have been indicated to be under the regulation of transcriptional control of SOX9. They include COL2A1, COL9A1, COL11A2, aggrecan, and cartilage link protein (CRTL1) genes (28–31), all of which are involved in articular cartilage structure and function. Furthermore, SOX9 is presented and present in presumptive cartilage during embryo development. The mutations in human SOX9 gene leads to campomelic dysplasia with skeletal malformation and dwarfism (32). Thus, downregulation of SOX9 in OA is clearly likely to contribute to cartilage pathology.

Moreover, induction of IGF1 150 ng/ml in WJMSCs also increased expression of COL2 (Figure 1). Referring to previous studies, SOX9 overexpression in human chondrocytes increases COL2A1 expression, as well as their capacity to reform a cartilage ECM (33–35). Collagenase-1, -2, and -3 are all synthesized by chondrocytes and have been considered as the rate-limiting enzymes in collagen degradation (36–38). Collagenase levels in the synovial fluid and serum correlate with cartilage destruction in OA (39). An imbalance between collagenases and their endogenous inhibitors has also been suggested to result in cartilage collagenolysis (40).

The results obtained in this study are in line with previous studies that proteoglycan core protein and collagen type II are induced by IGF1 and that it stabilizes the chondrocyte phenotype in pathological conditions (41–43). IGF-1 is fairly mitogenic in human adult articular cartilage and highly stimulates the production of chondrocyte extracellular matrix components (13, 14).

Furthermore, IGF1 with concentrations of 75 ng/ml and 120 ng/ml used to induce WJMSCs which were co-cultured with IL1β (5 ng/ml, 10 ng/ml)-induced CHON002. These IGF1 concentrations were used in this study because SOX9 expression is not appropriate. According to Kim and Im, 2009, supplementation of mesenchymal stem cells with IGF, did not induce
SOX9 significantly (44). Parameters measured were chondrogenic markers that included ADAMTS-5, ADAMTS-1, MMP-3, MMP-1, and RANKL. Elevation of MMP-1 (collagenase-1) and MMP-3 (stromelysin-1) have been documented in osteoarthritic cartilage (45, 46) and in the synovial fluid of osteoarthritic joints (47, 48). The present study has recognized the messenger RNA (mRNA) presence for some MMPs, i.e., MMP13, MMP9, MMP3, and MMP1, in human OA cartilage (49, 50), and other studies have reported specific MMP proteins and collagenase-mediated type II collagen degradation products (51, 52). These enzymes are involved in intrinsic chondrocyte-mediated degenerative changes of the cartilage matrix in OA.

In the present study, IGF-induced WJMSCs decreased the ADAMTS1 level. ADAMTS1 has shown in cartilage and the synovium (24) to cleave aggrecan and versican (53). Some studies reported that the expression of ADAMTS1 is significantly elevated in OA cartilage (25, 54–58), however, some studies also indicated a decreased expression in late-stage human OA (59–61).

The TNF family of molecules called RANKL (receptor activator of NFκB ligand), also known as osteoprotegerin ligand), osteoest differentiation factor (ODF), TNF related activation-induced cytokine (TRANCE), and TNFSF11 and its receptor RANK (TNFRSF11A) is the main regulator of bone remodeling and activation of osteoclasts (62, 63–66). In this study, IGF-induced WJMSCs also reduced the RANKL level. Production of RANKL activated by T-cells directly controls osteoclastogenesis, bone remodeling, and also associated with autoimmune diseases, cancers, leukemias, asthma, chronic viral infections, and periodontal disease (63). In particular, RANKL is more likely to be the pathogenetic principle that results in the destruction of bone and cartilage in arthritis. RANKL is highly presented in osteoblast/stromal cells, primitive mesenchymal cells surrounding the cartilaginous anlagen, and hypertrophying chondrocytes (62). RANKL mRNA has also been observed in hypertrophic and prehypertrophic chondrocytes at day 15 of embryogenesis and extraskeletal tissues such as the brain, heart, kidneys, skeletal muscles, and skin throughout mouse development (67). RANKL expression can be upregulated by bone-resorbing factors i.e., vitamin D3, glucocorticoids, IL1, IL6, IL11, IL17, TNFα, PGE2, and PTH (68, 62, 65).

These findings are supported by several studies. A study done by Ahmed et al. showed that both rat model and co-culture between MSCs and cartilage chips involve MMP-13 and tissue inhibitor of MMP1 and MMP2 as factors in hypertrophy (69). Moreover, some studies also reported enhanced chondrogenesis of MSCs co-cultured with chondrocytes, which shows higher cartilage-specific marker expression in the co-culture compared with mononucleotides as well as reduced expression of hypertrophic markers such as MMP3 (70, 71). IGF1-WJMSCs is, therefore, promising for use as medicine in the treatment of OA.

Conclusion

The IGF1-induced WJMSCs increased expression of COL2 and SOX9 compared to controls, which indicates IGF1-WJMSCs are capable of enhancing chondrogenesis and can be further used in OA treatment. Validation of IGF1-WJMSCs in animal models should eventually follow as further study.

Acknowledgment

This study was supported by the Grants-in-Aid from Insinas Riset Pratama 2017 and 2018, the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia. The authors like to thank Hanna Sari W Kusuma, Rahmawati Rahmawati, Risma Laila Qodariah, and Fajar Sukma Perdana from Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung, West Java, Indonesia for their technical assistance.

References

1. Bijlsma JW, Berenbaum F, Lafeber FP. Osteoarthritis: an update with relevance for clinical practice. Lancet 2010; 377: 2115-2126.
2. Anderson DD, Chubinskaya S, Guilak F. Post-traumatic osteoarthritis: improved understanding and opportunities for early intervention. J Orthop Res 2011; 29: 802-809.
3. Van den Berg WB. Osteoarthritis year 2010 in review: pathomechanisms. Osteoarthr Cartilage 2010; 19: 338-341.
4. Aigner T, Gebhard PM, Schmid E, Bau B, Harley V, Poschl E. SOX9 expression does no correlate with type II collagen expression in adult articular chondrocytes. Matrix Biol 2003; 22: 363-372.
5. Ashraf A, Cha BH, Kim JS, Ahn J, Han I, Park H, Lee SH. Osteoarthritis and Cartilage. Osteoarthritis Cartilage 2016; 24:196-205.
6. Buckwalter JA, Saltzman C, Brown T. The impact of osteoarthritis: implications for research. Clin Orthop Relat Res 2004; 56: 15.
7. Aigner T, McKenna L. Molecular pathology and pathobiology of osteoarthritic cartilage. Cell Mol Life Sci 2012; 59: 5-18.
8. Johnson K, Zhu S, Tremblay MS. A stem cell-based approach to cartilage repair. Science 2012; 336: 717-721.
9. Haleem-Smith H, Calderon R, Song Y. Cartilage oligomeric matrix protein enhances matrix assembly during chondrogenesis of human mesenchymal stem cells. J Cell Biochem 2012; 113: 1245-1252.
10. Hass R, Kasper C, Bohm S, Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): a comparison of adult and neonatal tissue-derived MSC. Cell Commun Signal 2011; 9: 12-25.
11. Widowati W, Laura W, Bachtier I. Effect of oxygen tension towards proliferation and characteristics of wharton’s jelly-derived mesenchymal stem cells. BGM 2014; 7: 1-8.
12. Kim HJ, Im GJ. Chondrogenic differentiation of adipose tissue-derived mesenchymal stem cells: Greater doses of growth factor are necessary. J Orthop Res 2009; 27: 612-619.
13. Guenther HL, Guenther HE, Froesch ER, Fleisch H. Effect of insulin-like growth factor on collagen and glycosaminoglycan synthesis by rabbit articular chondrocytes in culture. Experientia 1982; 38: 979-981.
14. McQuillan DJ, Handley CJ, Campbell MA, Bolis S, Milway VE, Herington AC. Stimulation of proteoglycan biosynthesis by serum and insulin-like growth factor-I in cultured bovine articular cartilage. Biochem J 1986; 240: 423-430.
15. Schoenen E, Zapf J, Humbel RE, Froesch ER. Insulin-like growth factor I stimulates growth in hypophysectomized rats. Nature 1982; 296: 252-253.
16. Trippel SB, Corvol MT, Dumontier MF, Rappaport R, Hung HH, Mankin HJ. Effect of somatotendin-C/insulin-like growth factor I and growth hormone on cultured growth plate and articular chondrocytes. Pediatr Res 1989; 25: 7-68-2.

17. Bhaumick B, Bala RM. Differential effects of insulin-like growth factors I and II on growth, differentiation and glucose regulation in differentiating chondrocyte cells in culture. Acta Endocrinol 1991; 125: 201-211.

18. Bhaumick B. Insulin-like growth factor (IGF) binding proteins and insulin-like growth factor secretion by cultured chondrocyte cells: Identification, characterization and ontogeny during cell differentiation. Regul Pept 1993; 48: 113-122.

19. Madry H, Kaul G, Guzziarini M, Stein U. Enhanced repair of articular cartilage defects in vivo by transplanted chondrocytes overexpressing insulin-like growth factor I (IGF-I). J Gene Ther 2005; 12: 1171-1179.

20. Widowati W, Wijaya L, Murti H, Widyastuti H, Agustina D, Laksmiawati DR, et al. Conditioned medium from normoxia (WJMSCs-normoCM) and hypoxia-treated WJMSCs (WJMSCs-hypoCM) in inhibiting cancer cell proliferation. Biomarkers and Genomic Medicine 2015; 7: 8-17.

21. Tsuchiya K, Chen G, Ushida T, Matsuno T, Tateishi T. The effect of coculture of chondrocytes with mesenchymal stem cells on their cartilaginous phenotype in vitro. Materials Science and Engineering: C 2004; 24: 391-396.

22. Ng Lj, Wheatley S, Muscat GE, Conway-Campbell J, Bowles J, Wright E. SOX9 binds DNA, activates transcription, and coexpresses with type II collagen during chondrogenesis in the mouse. Dev Biol 1997; 183: 108e21.

23. Zhao Q, Eberspaecher H, Lefebvre V, de Crombrugge B. Parallel expression of Sox9 and Col2a1 in cells undergoing chondrogenesis. Dev Dyn 1997; 209: 377e86.

24. Kelwick R, Desanlis I, Wheeler GN, Edwards DR. The ADAMTS (A Disintegrin and Metalloproteinase with Thrombospondin motifs) family. Genome Biol 2015; 16: 113-128.

25. Gardiner MD, Vincent TL, Driscoll P, Burleigh A, Bou-Gharios G, Saklatvala J. Transcriptional analysis of micro-dissected articular cartilage in post-traumatic murine osteoarthritis. Osteoarthritis and Cartilage 2015; 23: 616-628.

26. Seo S, Na K. Mesenchymal stem cell-based tissue engineering for the treatment of cartilage injury and cartilage disease. Stem Cells 2007; 25: 2886-2895.

27. Can A, Karahuseyinoglu S. Concise review: Human umbilical cord stroma with regard to the source of fetus–derived stem cells. Stem Cells Transl Med 2014; 3: 10744.

28. Sekiya I, Tsuji K, Koopman P, Watanabe H, Yamada Y, Cloutier JM, Pelletier JP. Excess of metalloproteinases over tissue inhibitor of metalloproteinase may contribute to cartilage degradation in osteoarthritis and rheumatoid arthritis. Lab Invest 1994; 70: 807-815.

29. Bhaumick B, Bassler C. Effects of hormones and local growth factors on articular chondrocyte metabolism. J Rheumatol 1991; 18 (Suppl 27): 68-70.

30. Sandell LJ, Dudek EJ. Insulin-like growth factor I stimulates type II collagen gene expression in cultured chondrocytes. J Rheumatol 1991; 18: 751-755.

31. Li Y, Tew SR, Russell AM, Gonzalez K, Hardingham TE, Hawkins RE. Transduction of human articular chondrocytes with adenoaviral, retroviral and lentiviral vectors and the effects of enhanced expression of SOX9. Tissue Eng 2004; 10: 575-584.

32. Tew SR, Li Y, Pothacharoen P, Tweets LM, Hawkins RE, Hardingham TE. Retroviral transduction with SOX9 enhances re-expression of the chondocyte phenotype in passaged osteoarthritic human articular chondrocytes. Osteoarthritis Cartilage 2005; 13: 80-89.

33. Franchimont P, Bassler C. Effects of hormones and local growth factors on articular chondrocyte metabolism. J Rheumatol 1991; 18 (Suppl 27): 68-70.

34. Sandell LJ, Dudek EJ. Insulin-like growth factor I stimulates type II collagen gene expression in cultured chondrocytes. Trans Orthop Res Soc 1988; 35: 300.

35. Tesch GH, Handley CJ, Cornell HJ, Herington AC. Effects of free and bound insulin-like growth factors on proteoglycan metabolism in articular cartilage explants. J Orthop Res 1992; 10: 14-22.

36. Kim HJ, Im GL. Chondrogenic Differentiation of Adipose Tissue-Derived Mesenchymal Stem Cells: Greater Doses of Growth Factor are Necessary. J Orthop Res 2009; 27:612-619.

37. Dean PW, Nelson JK, Schumacher J. Effects of age and prosthesis material on in vitro cartilage retention of laryngoplasty prostheses in horses. American Journal of Veterinary Research 1990; 51: 114-117.

38. Su S, Grover J, Roughley PJ, DiBattista JA, Martel-Pelletier J, Pelletier JP, et al. Expression of the tissue inhibitor of metalloproteinases (TIMP) gene family in normal and osteoarthritic joints. Rheumatology International 1999; 18: 183-191.

39. Clark IM, Powell LK, Ramsey S, Hazleman BL, Caswell TE. The measurement of collagenase, tissue inhibitor of metalloproteinases (timp), and collagenase—timp complex in synovial fluids from patients with osteoarthritis and
rheumatoid arthritis. Arthritis and Rheumatology 1993; 36: 372-379.
48. Cawston TE. Proteinases and inhibitors. British medical bulletin 1995; 51: 385-401.
49. Freemont AJ, Hampson V, Tilman R, Goupille P, Taiwo Y, Hoyland JA. Gene expression of matrix metalloproteinases 1, 3, and 9 by chondrocytes in osteoarthritic human knee articular cartilage is zone and grade specific. Annals of The Rheumatic Diseases 1997; 56: 542-548.
50. Shlopopov BV, Gumanovskaya ML, Hasty KA. Autocrine regulation of collagenase 3 (matrix metalloproteinase 13) during osteoarthritis. Arthritis and Rheumatism 2000; 43: 195-205.
51. Freemont AJ, Byers RJ, Taiwo YO, Hoyland JA. In situ zymographic localisation of type II collagen degrading activity in osteoarthritic human articular cartilage. Annals of the rheumatic diseases 1999; 58: 357-365.
52. Billinghamurst RC, Dahlberg L, Lonescu M, Reiner A, Bourne R, Rorabeck C. Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage. J Clin Invest 1997; 99: 1534-1545.
53. Rodríguez-Manzaneque JC, Westling J, Thai SN, Luque A, Knauper V, Murphy G. ADAMTS1 cleaves aggrecan at multiple sites and is differentially inhibited by metalloproteinase inhibitors. Biochem Biophys Res Commun 2002; 293: 501-508.
54. Geyer M, Grassel S, Straub RH, Schett G, Dinser R, Grifka J. Differential transcriptome analysis of intraarticular lesional vs intact cartilage reveals new candidate genes in osteoarthritis pathophysiology. Osteoarthritis and Cartilage, 2009; 17: 328-335.
55. Geyer M, Grassel S, Straub RH, Schett G, Dinser R, Grifka J. Differential transcriptome analysis of intraarticular lesional vs intact cartilage reveals new candidate genes in osteoarthritis pathophysiology. Osteoarthritis and Cartilage, 2009; 17: 328-335.
56. Karlsson C, Dehne T, Lindahl A, Brittberg M, Pruss A, Sittinger M. Genome-wide expression profiling reveals new candidate genes associated with osteoarthritis. Osteoarthritis and Cartilage, 2010; 18: 581-592.
57. Ramos YF, den Hollander W, Bovee JV, Bomer N, Van der Breggen R, Lakenberg N. Genes involved in the osteoarthritis candidate genes associated with osteoarthritis. Osteoarthritis and Cartilage, 2010; 18: 581-592.
58. Wachsmuth L, Bau B, Fan Z, Pecht A, Gerwin N, Aigner T. ADAMTS-1, a gene product of articular chondrocytes in vivo and in vitro, is downregulated by interleukin 1beta. J Rheumatol 2004; 31: 315-320.
59. Davidson RK, Waters JG, Kevorkian L, Darrah C, Cooper A, Donell ST. Expression profiling of metalloproteinases and their inhibitors in synovium and cartilage. Arthritis Res Ther 2006; 8: R124-R133.
60. Kevorkian L, Young DA, Darrah C, Donell ST, Shepstone L, Porter S. Expression profiling of metalloproteinases and their inhibitors in cartilage. Arthritis Rheum 2004; 50: 131-141.
61. Swingler TE, Waters JG, Davidson RK, Pennington CJ, Puente XS, Darrah C. Degradome expression profiling in human articular cartilage. Arthritis Res Ther 2009; 11: R96.
62. Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 1998; 93: 165-176.
63. Kong YY, Feige U, Sarosi I, Bolon B, Tafuri A, Morony S. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 1999; 402: 304-309.
64. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Lukhy R. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997; 99: 309-319.
65. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinoshita M, Mochizuki S. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA 1998; 95: 3597-3602.
66. Wong BR, Rho J, Arron J, Robinson E, Orlinick J, Chao M. TRANCE is a novel ligand of the tumor necrosis factor receptor family that activates c-Jun N-terminal kinase in T cells. J Biol Chem 1997; 272: 25190-25194.
67. Kartsogiannis V, Zhou H, Horwood NJ, Thomas RJ, Hards DK, Quinn J. Localization of RANKL (receptor activator of NFkB ligand) mRNA and protein in skeletal and extraskeletal tissues. Bone 1999; 25: 525-534.
68. Roodman GD. Cell biology of the osteoclast. Exp Hematol. 1999; 27: p. 1229-1241.
69. Ahmed N, Dreier R, Göpferich A, Grifka J, Grässel S. Soluble signalling factors derived from differentiated cartilage tissue affect chondrogenic differentiation of rat adult marrow stromal cells. Cell Physiol Biochem 2007; 20: 665-678.
70. Bian L, Zhai DY, Mauck RL, Burdick JA. Coculture of human mesenchymal stem cells and articular chondrocytes reduces hypertrophy and enhances functional properties of engineered cartilage. Tissue Eng A 2011; 17: 1137-1145.
71. Babur BK, Kabiri M, Klein TJ, Lott WB, Doran MR. The rapid manufacture of uniform composite multicellular-biomaterial microspheres, their assembly into macroscopic organized tissues, and potential applications in cartilage tissue engineering. PLoS One 2015; 10: e0122250.