RESEARCH ARTICLE

Sclerostin inhibits interleukin-1β-induced late stage chondrogenic differentiation through downregulation of Wnt/β-catenin signaling pathway

Kazuma Miyatake, Ken Kumagai*, Sosuke Imai, Yasuteru Yamaguchi, Yutaka Inaba

Department of Orthopaedic Surgery and Musculoskeletal Science, Graduate School of Medicine, Yokohama City University, Yokohama, Japan

* kumagai@yokohama-cu.ac.jp

Abstract

It is known that Wnt/β-catenin signaling induces endochondral ossification and plays a significant role in the pathophysiology of osteoarthritis (OA). Sclerostin is a potent inhibitor of the Wnt/β-catenin signaling pathway. This study investigated the role of sclerostin in the endochondral differentiation under an OA-like condition induced by proinflammatory cytokines. ATDC5 cells were used to investigate chondrogenic differentiation and terminal calcification, and 10 ng/ml IL-1β and/or 200 ng/ml sclerostin were added to the culture medium. IL-1β impaired early chondrogenesis from undifferentiated state into proliferative chondrocytes, and it was not altered by sclerostin. IL-1β induced progression of chondrogenic differentiation in the late stage and promoted terminal calcification. These processes were inhibited by sclerostin and chondrogenic phenotype was restored. In addition, sclerostin restored IL-1β-induced upregulation of Wnt/β-catenin signaling in the late stage. This study provides insights into the possible role of sclerostin in the chondrogenic differentiation under the IL-1β-induced OA-like environment. Suppression of Wnt signaling by an antagonist may play a key role in the maintenance of articular homeostasis and has a potential to prevent the progression of OA. Thus, sclerostin is a candidate treatment option for OA.

Introduction

Osteoarthritis (OA) is a degenerative joint disease, characterized by cartilage degradation, subchondral bone sclerosis, osteophyte formation, and synovial inflammation. A complex network of multifactorial mechanisms including biochemical, mechanical, and enzymatic aspects are involved in the pathogenesis of OA [1]. Proinflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α are the critical mediators of the disturbed processes implicated in OA pathophysiology [2].

The Wnt/β-catenin signaling pathway plays a significant role in the pathophysiology of OA [3]. A previous study demonstrated that inhibition of Wnt/β-catenin signaling by small molecules can effectively prevent IL-1β- and TNFα-induced cartilage degradation by blocking the
production of matrix metalloproteinase (MMP) [4]. Furthermore, tissue-specific activation of Wnt/β-catenin signaling in articular chondrocytes of adult mice resulted in progressive loss of articular cartilage and an OA-like phenotype [5]. Thus, blockade of Wnt/β-catenin signaling may be proposed as a therapeutic target.

Sclerostin, encoded by the SOST gene, is known to be one of the Wnt signaling antagonists [6]. Sclerostin regulates disease processes in OA by opposing the effects of promotion of disease-associated subchondral bone sclerosis, while inhibiting the degradation of cartilage [7]. The deficiency of SOST aggravates the OA phenotype by increasing catabolic activity of cartilage [8], and SOST-knockout mice exhibited severe progression of OA in response to joint instability, suggesting that sclerostin may contribute to the maintenance of cartilage integrity in OA [9]. However, effects of sclerostin on terminal calcification of chondrocytes in the osteoarthritic environment are unknown and need to be elucidated, considering that endochondral ossification signals may be important for OA progression [10].

We previously demonstrated that SOST is upregulated in the early stage of chondrogenic differentiation, but is not required for endochondral ossification [11]. This study focused on the role of sclerostin in the chondrogenic differentiation under the OA-like condition induced by proinflammatory cytokines. We hypothesized that sclerostin upregulates chondrogenic differentiation to proliferating chondrocytes and downregulates endochondral ossification under the proinflammatory cytokine-induced condition. This study investigated IL-1β-induced osteochondral differentiation in vitro, and examined whether sclerostin can restore the chondrogenic phenotype.

Materials and methods
Cell lines and culture conditions
ATDC5 cells (mouse embryo teratocarcinoma-derived chondrogenic cell line) were purchased from European Collection of Cell Cultures (ECACC, Public Health England, Porton Down, UK), and were cultured at a seeding density of 4 × 10⁴ cells/well for a 12-multiwell plate, 6 × 10⁴ cells/well for a 6-multiwell plate and 7 × 10⁴ cells/well for a 8-multiwell chamber slide, in a 1:1 mixture of Dulbecco’s modified Eagle’s and Ham’s F12 medium (Flow Laboratories, Irvine, UK) supplemented with 5% fetal bovine serum (FBS: GIBCO, New York, NY, USA), 10 μg/ml bovine insulin (I; Wako Pure Chemical, Osaka, Japan), 10 μg/ml human transferrin (T; Boehringer Mannheim, Mannheim, Germany), and 3 × 10⁻⁸ M sodium selenite (S; Sigma Chemical Co., St. Louis, MO, USA) at 37°C in a humidified atmosphere of 5% CO₂ in air for the initial 3 weeks, as previously described [10, 15]. On day 21, the culture medium was switched to alpha modified essential medium supplemented with 5% FBS plus ITS, and the CO₂ concentration was shifted to 3% to facilitate mineralization, as previously described [10, 15]. The medium was replaced every other day. To characterize the cells that are not subjected to chondrogenic media, ATDC5 cells were also cultured without ITS (S1 Fig). For the early stage experiment, IL-1β and/or sclerostin were filled in each well from 3 days to 3 weeks. For the late stage experiment, IL-1β and/or sclerostin were filled in each well from 3 weeks to 7 weeks. To mimic the OA-like condition, 10 ng/ml recombinant murine IL-1β (PeproTech, Rocky Hill, NJ, USA) was used, as previously described [12–14]. The effect of sclerostin was examined by the addition of 200 ng/ml recombinant mouse SOST (R&D systems).

Alcian blue staining
To visualize the deposition of sulfated glycosaminoglycan (sGAG), a marker for chondrogenic differentiation, cells were fixed with 100% methanol and stained with 0.1% Alcian blue 8GS (Sigma) in 0.1 N HCl for 4 h at room temperature, as previously described [10].
sGAG assay

The culture media were collected and sGAG content was quantified using a commercially available sGAG Alcian blue binding assay kit (Euro-Diagnostica, Malmo, Sweden). The absorbance at 640 nm was measured using a microplate reader (Infinite F50, TECAN, Kawasaki, Japan), as previously described [10].

Alizarin red staining

To evaluate visualize the calcium deposits, cells were fixed with phosphate-buffered formalin and then stained with 40 mM alizarin red S (pH 4.2, Sigma) for 30 min, as previously described [10]. The Alizarin red-stained areas were scanned using an image scanner and analyzed qualitatively using Image J software.

Immunostaining

The cultured cells were washed one time with cold PBS and fixed with 4% paraformaldehyde for 10 minutes at room temperature. Following washing three times with PBS, the cells were permeabilized with 0.2% Triton X-100 in PBS buffer for 20 minutes at room temperature. Following washing three times with 0.1% Tween20 in PBS, the cells were blocked with 0.1% Tween20 and 10% goat serum, with 1% BSA in PBS buffer for 1 hour at room temperature. The cells were incubated with the anti-rabbit primary antibodies of β-catenin (Abcam, Cambridge, UK), Axin1 (Novus Biologicals, Centennial, USA), Axin2 (Novus Biologicals) and phosphorylated LRP6 (Biorbyt Ltd., Cambridge, UK) over night at 4˚C. The cells were washed three times with 0.1% Tween20 in PBS. The cells were incubated with Alexa Fluor® 568 conjugated goat anti-rabbit IgG secondary antibody (Invitrogen, Carlsbad, USA) for 45 minutes at room temperature. To visualize the nuclei, the cells were double-stained with 4’, 6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Burlingame, USA). The cells were viewed with a Keyence BZ 800 epifluorescence microscope, which was equipped with a digital camera (CFI 60, Nikon Corporation, Tokyo, Japan). All immunofluorescence images were obtained with identical exposure settings.

Total RNA isolation and real-time RT-PCR

As described in detail previously [10], mRNA expression levels were analyzed following procedures. Total RNA was extracted from the cultured cells using Trizol reagent according to the manufacturer’s instructions (Invitrogen, Carlsbad, CA, USA). RNA was quantified by measuring absorbance at 260 nm, and the quality was confirmed by 260/280 nm absorbance ratio greater than 1.8. First-strand cDNA synthesis from total RNA was performed using an iScript™ advanced cDNA synthesis kit (BIO-RAD, Richmond, CA, USA). Quantitative real-time PCR was carried out using TaqMan gene expression assays (Applied Biosystems, Foster City, CA, USA) on a CFX96™ real-time PCR detection system (BIO-RAD). Expression of the gene of interest was normalized to GAPDH expression. TaqMan gene expression assays used in this study were as follows: Col2a1 (Mm01309565_m1); Col10a1 (Mm00487041_m1); Sox9 (Mm00448840_m1); Runx2 (Mm00501584_m1); BMP2 (Mm01340178_m1); Wnt3a (Mm03053669_s1); Wnt5a (Mm00437347_m1); LRP5 (Mm01227476_m1); LRP6 (Mm00999795_m1); Axin1 (Mm01299060_m1); Axin2 (Mm00443610_m1); Ctnnb1 (β-catenin) (Mm00499427_m1); MMP13 (Mm00439491_m1); ADAMTS5 (Mm00478620_m1); and GAPDH (Mm99999915_g1).
Statistical analysis
All experiments were repeated independently at least three times. All data are presented as means ± standard deviation. The analysis was performed with JMP Pro 12 software (SAS Institute Inc.) for Mac. Continuous variables were expressed as means. One-way analysis of variance (ANOVA) was used to compare mean values from different samples. Tukey’s HSD was used for post-hoc analyses. A value of $P < 0.05$ was considered significant.

Results

No restorative effects of sclerostin on IL-1β-induced impairment in the early stage of chondrogenic differentiation
To simulate the inflammatory environment, 10 ng/ml IL-1β was added to the culture media for chondrogenic differentiation, and the effects of sclerostin were assessed. The mRNA expressions of markers for chondrogenic differentiation were significantly decreased with IL-1β treatment, and they were not restored by sclerostin (Fig 1a). The mRNA expression of MMP-13, one of markers for cartilage catabolism, was significantly increased with IL-1β treatment, and they were not significantly altered by sclerostin (Fig 1b). Chondrogenic differentiation with proteoglycan synthesis was confirmed by positive staining with Alcian blue after 3 weeks (Fig 1c). Less intense staining was observed with the addition of IL-1β, and it was not restored by sclerostin. The expression of Wnt/β-catenin-associated genes, Wnt5a, LRP5, LRP6, Axin1, Axin2, and Ctnb-1, was significantly decreased with IL-1β treatment, and was not restored by sclerostin (Fig 2a). Less intense expressions of Axin1, Axin2, and β-catenin was observed in the immunofluorescence images of the cultured cells with the addition of IL-1β, and these were not restored by sclerostin (Fig 2b–2d). These results suggested that IL-1β induces impairment in the early stage of chondrogenic differentiation and downregulation of the Wnt/β-catenin signaling. In this condition, there are no restorative effects of sclerostin on IL-1β-induced impairment in the early stage of chondrogenic differentiation.

Inhibitory effects of sclerostin on IL-1β-induced terminal calcification in the late stage of chondrogenic differentiation
To assess the effects of sclerostin on IL-1β-induced terminal calcification in the late stage of chondrogenic differentiation, IL-1β and sclerostin were added from 3 weeks of culture. The mRNA expression of SOST was significantly decreased by IL-1β (S2 Fig). The mRNA expressions of markers for chondrogenic differentiation were significantly decreased with IL-1β, but they were restored by sclerostin (Fig 3a). The mRNA expressions of markers for cartilage catabolism and BMP2 were significantly increased with IL-1β, but they were restored by sclerostin (Fig 3b and 3c). Terminal calcification was confirmed by positive staining with Alizarin red after 7 weeks (Fig 3d). Stronger staining was identified with addition of IL-1β, but it was inhibited by sclerostin. The expression of Wnt/β-catenin associated genes, Wnt3a, Wnt5a, LRP6, Axin1, and Ctnb-1, was significantly increased with IL-1β, and was restored by sclerostin (Fig 4a). The immunofluorescence images of the cultured cells with the addition of IL-1β showed more intense expressions of phosphorylated LRP6, Axin1, Axin2, and β-catenin, but these were diminished by sclerostin (Fig 4b–4e). These results suggested that IL-1β promotes cartilage degradation and terminal calcification by upregulating the Wnt/β-catenin signaling pathway, and those effects were inhibited by sclerostin.
Discussion

The most important findings of this study were that sclerostin restores the chondrogenic phenotype and inhibits endochondral ossification under the IL-1β-induced condition. This process was associated with the downregulation of Wnt/β-catenin signaling. However, sclerostin did not alter the IL-1β-impaired early chondrogenesis from the undifferentiated state into
Fig 2. Effect of sclerostin on Wnt/β-catenin signaling pathway in early stage of chondrogenic differentiation with IL-1β. (A) Relative mRNA expressions of LRP5, LRP6, Axin1, Axin2, Wnt3a, Wnt5a, and Ctnb-1. N = 4 *P<0.05. (B-D) Immunofluorescence images of the cultured cells expressing Axin1 (B), Axin2 (C), and β-catenin (D). Scale bars = 100 μm.

https://doi.org/10.1371/journal.pone.0239651.g002
proliferative chondrocytes. The major advance in the present study is the investigation of the restorative effects of sclerostin on chondrogenic differentiation in the multistep process from mesenchymal chondroprogenitor to terminal calcification in vitro, which can be separately assessed during the early and late stages of chondrogenic differentiation.

Fig 3. Effect of sclerostin on late stage of chondrogenic differentiation in the presence of IL-1β. ATDC5 cells were cultured for chondrogenic conditions, and 10 ng/ml IL-1β and/or 200 ng/ml sclerostin were added to the culture medium from 21 days. (A) Relative mRNA expressions of markers for chondrogenic differentiations, Sox9, Runx2, Col2a1, and Col10a. (B) Relative mRNA expressions of markers for cartilage catabolism, VEGF, MMP13, and Adamts5. (C) Relative mRNA expression of BMP2. (D) Alizarin red staining of ATDC5 cells under condition of endochondral ossification at 7 weeks of culture (left). Relative staining area indicating total size of calcified nodules (right). N = 4 * P < 0.05.

https://doi.org/10.1371/journal.pone.0239651.g003
Fig 4. Effect of sclerostin on Wnt/β-catenin signaling pathway in late stage of chondrogenic differentiation with IL-1β. (A) Relative mRNA expressions of LRP5, LRP6, Axin1, Axin2, Wnt3a, Wnt5a, and Ctnb-1. N = 4 *P<0.05. (B-E) Immunofluorescence images of the cultured cells expressing phosphorylated LRP6 (B), Axin1 (C), Axin2 (D), and β-catenin (E). Scale bars = 100 μm.

https://doi.org/10.1371/journal.pone.0239651.g004
As described in detail previously [15, 16], ATDC5 is a good model system for studying the dynamic processes of chondrogenesis, and many findings in this system may have relevance to chondrogenesis in vivo. In our previous study, the role of sclerostin as an inhibitor of the canonical Wnt signaling pathway in the chondrogenic differentiation could be characterized using the same model system [11]. This established model system was used to investigate the effects on chondrogenic differentiation at the different timing of early and later stages in the present study. Although several in vivo OA models have been developed to investigate the pathological feature and therapeutic effects, they need to be considered influence under the multifactorial and complex conditions including mechanical load, synovial inflammation, cartilage degeneration and abnormal bone remodeling. This study simply focused the effect of sclerostin on the cytokine among several factors associated with OA pathogenesis.

The process of endochondral ossification, including chondrocyte hypertrophy, production of proteinases and cartilage apoptosis, is thought to be involved in the initiation and progression of OA [10, 17]. Wnt/β-catenin signaling plays a key role in the development of endochondral ossification and regulates OA development [3, 5]. As described in detail previously [3], β-catenin-dependent canonical Wnt signaling is required for the progression of endochondral ossification and growth of axial and appendicular skeletons, while excessive activation of this signaling can cause severe inhibition of initial cartilage formation and growth plate organization and function. Increased canonical Wnt signaling inhibits chondrogenesis [18, 19], but once cartilage has formed, it promotes chondrocyte maturation, enhances perichondral bone formation, initiates cartilage vascularization, and drives the formation of primary and secondary ossification centers [20]. Sclerostin is an inhibitor of Wnt/β-catenin signaling, which is expressed in the chondrocyte and modulates chondrogenic differentiation [11]. Thus, the present study focused on sclerostin as a potential target for the suppression of OA.

The present study investigated the restorative effects of sclerostin on chondrogenic differentiation under the IL-1β-induced condition in the different timing. IL-1β is a primary mediator of local inflammatory processes in OA [21, 22], and IL-1β-induced degradation of chondrogenesis is often utilized for in vitro model of OA [23, 24]. Since IL-1β is not cytotoxic up to a concentration of 100 ng/mL [12], and 10 ng/mL IL-1β is considered as an optimized concentration to induce OA-like condition in vitro, 10 ng/mL of concentration was used in this study [13, 14]. IL-1β modulates the chondrogenic differentiation, and those effects differ between the early and late stages. In the early stage, IL-1β downregulated Wnt/β-catenin signaling and impaired chondrogenic differentiation. In this condition, Wnt/β-catenin signaling has already been inhibited, and there was no further requirement for sclerostin to act as Wnt inhibitor. In addition, IL-1β downregulates the synthesis of master chondrogenic factor Sox9 [25], and inhibits early differentiation from mesenchymal phenotype into proliferative chondrocytes [26, 27]. In contrast, IL-1β promotes endochondral ossification with increased expressions of catabolic markers in the later stage [28, 29]. Although details of IL-1β and Wnt cross talk have not been well elucidated, as a possible mechanism, it was reported that nitric oxide mediates the IL-1β-induced inflammatory response of chondrocytes through the upregulation of Wnt signaling [30]. In the present study, sclerostin inhibited the IL-1β-induced terminal calcification through downregulation of Wnt/β-catenin signaling. Thus, the restorative effects of sclerostin on chondrogenic phenotype under the IL-1β-induced condition may be expected only in the later stage of chondrogenic differentiation.

In summary, the present study demonstrated that sclerostin restores the chondrogenic phenotype and inhibits endochondral ossification under the IL-1β-induced OA-like environment. IL-1β reduces the production of the Wnt antagonist, and enhances Wnt signaling in the articular resident cells [31]. Suppression of the Wnt signaling by the antagonist may play a key role...
in the maintenance of articular homeostasis [9, 32], and has the potential to prevent the progression of OA. Thus, sclerostin is a candidate treatment option for OA.

Supporting information

S1 Fig. To characterize the cells that are not subjected to chondrogenic media, ATDC5 cells were cultured with or without ITS. (A) Alcian Blue staining of ATDC5 cells at 3 weeks of culture in chondrogenic media (left) and non-chondrogenic media (right). Less intense staining is observed in non-chondrogenic media. Scale bars = 100 μm. (B) Alizarin red staining of ATDC5 cells at 7 weeks of culture in chondrogenic media (left) and non-chondrogenic media (right). Less intense staining is observed in non-chondrogenic media. Scale bars = 100 μm. (TIFF)

S2 Fig. To assess the effect of IL-1β on expression of sclerostin, ATDC5 cells were cultured for 3 weeks in chondrogenic media and then 10 ng/ml IL-1β was added to the media for 24 hours. The relative mRNA expression of SOST is significantly decreased in the cells with addition of IL-1β. N = 4 *P<0.05. (TIFF)

Author Contributions

Conceptualization: Ken Kumagai.
Data curation: Kazuma Miyatake, Yasuteru Yamaguchi.
Formal analysis: Kazuma Miyatake, Ken Kumagai, Yasuteru Yamaguchi.
Funding acquisition: Ken Kumagai, Yutaka Inaba.
Investigation: Kazuma Miyatake, Ken Kumagai, Sosuke Imai, Yasuteru Yamaguchi.
Methodology: Kazuma Miyatake, Ken Kumagai, Yasuteru Yamaguchi.
Supervision: Ken Kumagai.
Writing – original draft: Kazuma Miyatake, Ken Kumagai.
Writing – review & editing: Sosuke Imai, Yasuteru Yamaguchi, Yutaka Inaba.

References

1. Hunter DJ, Bierma-Zeinstra S. Osteoarthritis. Lancet. 2019; 393: 1745–1759. https://doi.org/10.1016/S0140-6736(19)30417-9 PMID: 31034380
2. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nat Rev Rheumatol. 2011; 7: 33–42. https://doi.org/10.1038/nrrheum.2010.196 PMID: 21119608
3. Usami Y, Gunawardena AT, Iwamoto M, Enomoto-Iwamoto M. Wnt signaling in cartilage development and diseases: lessons from animal studies. Lab Invest. 2016; 96: 186–96. https://doi.org/10.1038/labinvest.2015.142 PMID: 26641070
4. Landman EB, Miclea RL, van Blitterswijk CA, Karperien M. Small molecule inhibitors of WNT/beta-catenin signaling block IL-1beta- and TNFalpha-induced cartilage degradation. Arthritis Res Ther. 2013; 15: R93. https://doi.org/10.1186/ar4273 PMID: 23965253
5. Zhu M, Tang D, Wu Q, Hao S, Chen M, Xie C, et al. Activation of beta-catenin signaling in articular chondrocytes leads to osteoarthritis-like phenotype in adult beta-catenin conditional activation mice. J Bone Miner Res. 2009; 24: 12–21. https://doi.org/10.1359/jbmr.080901 PMID: 18767925
6. Semenov M, Tamaki K, He X. SOST is a ligand for LRPS/LRP6 and a Wnt signaling inhibitor. J Biol Chem. 2005; 280: 26770–5. https://doi.org/10.1074/jbc.M504308200 PMID: 15908424
7. Chan BY, Fuller ES, Russell AK, Smith SM, Smith MM, Jackson MT, et al. Increased chondrocyte sclerostin may protect against cartilage degradation in osteoarthritis. Osteoarthritis Cartilage. 2011; 19: 874–85. https://doi.org/10.1016/j.joca.2011.04.014 PMID: 21619935

8. Li J, Xue J, Jing Y, Wang M, Shu R, Xu H, et al. SOST Deficiency Aggravates Osteoarthritis in Mice by Promoting Sclerosis of Subchondral Bone. Biomed Res Int. 2019; 2019: 7623562. https://doi.org/10.1155/2019/7623562 PMID: 31828128

9. Bouaziz W, Funck-Brentano T, Lin H, Marty C, Ea HK, Hay E, et al. Loss of sclerostin promotes osteoarthritis in mice via beta-catenin-dependent and -independent Wnt pathways. Arthritis Res Ther. 2015; 17: 24. https://doi.org/10.1186/s13075-015-0540-6 PMID: 25656376

10. Kawaguchi H. Endochondral ossification signals in cartilage degradation during osteoarthritis progression in experimental mouse models. Mol Cells. 2008; 25: 1–6. PMID: 18319608

11. Yamaguchi Y, Kumagai K, Imai S, Miyatake K, Saito T. Sclerostin is upregulated in the early stage of chondrogenic differentiation, but not required in endochondral ossification in vitro. PLoS One. 2018; 13: e0201839. https://doi.org/10.1371/journal.pone.0201839 PMID: 30071108

12. Graeser AC, Giller K, Wiegand H, Barella L, Boesch Saadatmandi C, Rimbach G. Synergistic chondroprotective effect of alpha-tocopherol, ascorbic acid, and selenium as well as glucosamine and chondroitin on oxidant induced cell death and inhibition of matrix metalloprotease-3—studies in cultured chondrocytes. Molecules. 2009; 15: 27–39. https://doi.org/10.3390/molecules15010027 PMID: 20110869

13. Shi S, Man Z, Li W, Sun S, Zhang W. Silencing of Wnt5a prevents interleukin-1beta-induced collagen type II degradation in rat chondrocytes. Exp Ther Med. 2016; 12: 3161–3166. https://doi.org/10.3892/etm.2016.3788 PMID: 27882132

14. Wang F, Liu J, Chen X, Zheng X, Qu N, Zhang B, et al. IL-1b receptor antagonist (IL-1Ra) combined with autophagy inducer (TAT-Beclin1) is an effective alternative for attenuating extracellular matrix degradation in rat and human osteoarthritis chondrocytes. Arthritis Res Ther. 2019; 21: 171. https://doi.org/10.1186/s13075-19-1952-5 PMID: 31291980

15. Kondo S, Shukunami C, Morioka Y, Matsumoto N, Takahashi R, Oh J, et al. Dual effects of the membrane-anchored MMP regulator RECK on chondrogenic differentiation of ATDC5 cells. J Cell Sci. 2007; 120: 849–57. https://doi.org/10.1242/jcs.03388 PMID: 17298979

16. Shukunami C, Ishizeki K, Atsumi T, Ohta Y, Suzuki F, Hiraki Y. Cellular hypertrophy and calcification of embryonal carcinoma-derived chondrogenic cell line ATDC5 in vitro. J Bone Miner Res. 1997; 12: 1174–88. https://doi.org/10.1002/jbmr.1997.12.8.1174 PMID: 9258747

17. Saito T, Fukai A, Mabuchi A, Ikeda T, Yano F, Ohba S, et al. Transcriptional regulation of endochondral ossification by HIF-2alpha during skeletal growth and osteoarthritis development. Nat Med. 2010; 16: 678–86. https://doi.org/10.1038/nm.2146 PMID: 20495570

18. Day TF, Guo X, Garrett-Beal L, Yang Y. Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Dev Cell. 2005; 8: 739–50. https://doi.org/10.1016/j.devcel.2005.03.016 PMID: 15866164

19. Hill TP, Spater D, Taketo MM, Birchmeier W, Hartmann C. Canonical Wnt/beta-catenin prevents osteoblasts from differentiating into chondrocytes. Dev Cell. 2005; 8: 727–38. https://doi.org/10.1016/j.devcel.2005.02.013 PMID: 15866163

20. Dao DY, Jonason JH, Zhang Y, Hsu W, Chen D, Hilton MJ, et al. Cartilage-specific beta-catenin signaling regulates chondrocyte maturation, generation of ossification centers, and perichondrial bone formation during skeletal development. J Bone Miner Res. 2012; 27: 1680–94. https://doi.org/10.1002/jbmr.1639 PMID: 22508079

21. Daheesha M, Yao QJ. The interleukin-1beta pathway in the pathogenesis of osteoarthritis. J Rheumatol. 2008; 35: 2306–12. https://doi.org/10.3899/jrheum.080346 PMID: 18925684

22. Jenei-Lanzi Z, Meurer A, Zaucke F. Interleukin-1beta signaling in osteoarthritis—chondrocytes in focus. Cell Signal. 2019; 53: 212–223. https://doi.org/10.1016/j.cellsig.2018.10.005 PMID: 30312659

23. Goldring SR, Goldring MB. The role of cytokines in cartilage matrix degeneration in osteoarthritis. Clin Orthop Relat Res. 2004; S27–36. https://doi.org/10.1097/01.blo.0000144853.66566.8f PMID: 15480070

24. Johnson CI, Argyle DJ, Clements DN. In vitro models for the study of osteoarthritis. Vet J. 2016; 209: 40–9. https://doi.org/10.1016/j.tvjl.2015.07.011 PMID: 26831151

25. Murakami S, Lefebvre V, de Crombrugghe B. Potent inhibition of the master chondrogenic factor Sox9 gene by interleukin-1 and tumor necrosis factor-alpha. J Biol Chem. 2000; 275: 3687–92. https://doi.org/10.1074/jbc.275.5.3687 PMID: 10652367
26. Simsa-Maziel S, Monsonego-Ornan E. Interleukin-1beta promotes proliferation and inhibits differentiation of chondrocytes through a mechanism involving down-regulation of FGFR-3 and p21. Endocrinology. 2012; 153: 2296–310. https://doi.org/10.1210/en.2011-1756 PMID: 22492305

27. Wehling N, Palmer GD, Pilapil C, Liu F, Wells JW, Muller PE, et al. Interleukin-1beta and tumor necrosis factor alpha inhibit chondrogenesis by human mesenchymal stem cells through NF-kappaB-dependent pathways. Arthritis Rheum. 2009; 60: 801–12. https://doi.org/10.1002/art.24352 PMID: 19248089

28. Mumme M, Scotti C, Papadimitropoulos A, Todorov A, Hoffmann W, Bocelli-Tyndall C, et al. Interleukin-1beta modulates endochondral ossification by human adult bone marrow stromal cells. Eur Cell Mater. 2012; 24: 224–36. https://doi.org/10.22203/ecm.v024a16 PMID: 23007908

29. Scotti C, Piccinini E, Takizawa H, Todorov A, Bourgine P, Papadimitropoulos A, et al. Engineering of a functional bone organ through endochondral ossification. Proc Natl Acad Sci U S A. 2013; 110: 3997–4002. https://doi.org/10.1073/pnas.1220108110 PMID: 23401508

30. Zhong L, Schivo S, Huang X, Leijten J, Karperien M, Post JN. Nitric Oxide Mediates Crosstalk between Interleukin 1beta and WNT Signaling in Primary Human Chondrocytes by Reducing DKK1 and FRZB Expression. Int J Mol Sci. 2018; 1945–1954. https://doi.org/10.1007/s10753-018-0838-z PMID: 29956067

31. Yoshida Y, Yamasaki S, Oi K, Kuranobu T, Nojima T, Miyaki S, et al. IL-1beta Enhances Wnt Signal by Inhibiting DKK1. Inflammation. 2018; 41: 1945–1954. https://doi.org/10.1007/s10753-018-0838-z PMID: 29956067

32. Wu J, Ma L, Wu L, Jin Q. Wnt-beta-catenin signaling pathway inhibition by sclerostin may protect against degradation in healthy but not osteoarthritic cartilage. Mol Med Rep. 2017; 15: 2423–2432. https://doi.org/10.3892/mmr.2017.6278 PMID: 28259981