Stimulation of Chondrogenic Differentiation of Mesenchymal Stem Cells

Da-Ae Yu, Jin Han, Byung-Soo Kim

School of Chemical and Biological Engineering, Seoul National University, Seoul, Korea

The methods for cartilage repair have been studied so far, yet many of them seem to have limitations due to the low regenerative capacity of articular cartilage. Mesenchymal stem cell (MSC) has been suggested as an alternative solution to remedy this challenging problem. MSCs, which have extensive differentiation capacity, can be induced to differentiate into chondrocytes under specific conditions. Particularly, this review focused on the effects of growth factors, cell-to-cell interactions and biomaterials in chondrogenesis of MSCs. Appropriate stimulations through these factors are crucial in differentiation and proliferation of MSCs. However, use of MSCs for cartilage repair has some drawbacks and risks, such as expression of hypertrophy-related genes in MSCs-derived chondrocytes and consequent calcification or cell death. Nevertheless, the clinical application of MSCs is expected in the future with advanced technology.

Keywords: Mesenchymal stem cell, Chondrogenesis, Growth factor, Cellular interaction, Biomaterial

Introduction

Articular cartilage, a connective tissue comprising main part of joints, enables smooth movement of the joints by reducing the frictional stresses between bones. This characteristic is mainly influenced by the excessive portion of water and extracellular matrices (ECMs), mostly collagen type II, consequently forming swelling structure. Collagens attribute to the shape and strength of cartilage, while proteoglycans give resistance to compression. However, these constituents of articular cartilage have long half-lives and their turn-over or replacement is very low once they are formed. Also, lack of blood vessels in cartilage makes it difficult to transfer progenitor cells, nutrients, and growth factors (GFs) into the articular tissue, consequently resulting in difficulty of repair (1).

Several methods for cartilage repair have been developed, but many of them have limitations including donor site morbidity, fibrocartilage formation and leakage of implanted cells. Even after successful implantation of chondrocytes, maintenance of chondrogenic phenotype is difficult and the cells are prone to turn into fibrous cartilage. Additionally, insufficient segregation of the cartilaginous matrix from these implanted chondrocytes poses another limitation for cartilage repair. Implantation and chondrogenesis of MSCs may be a solution to overcome these problems. MSCs have unique properties, such as self-renewal, extensive proliferation and differentiation into multilineages (2). Also, an efficient expansion capability of MSCs *in vitro* serves these cells as a great candidate for future cartilage repair component. Results from a few animal studies indicated that tissues repaired with MSCs showed better cell arrangement and integration with surroundings than those repaired with chondrocytes. Moreover, they can be extracted from various adult mesenchymal tissues, such as bone marrow, peripheral blood and adipose tissues. Chondrogenesis of MSCs could be achieved by modulating MSC interactions with cell microenvironments, such as GFs, neighboring cells, and cell-ad-

Accepted for publication February 10, 2012

Correspondence to **Byung-Soo Kim**

School of Chemical and Biological Engineering, Seoul National University, 1, Gwanak-ro, Gwanak-gu, Seoul 151-742, Korea

Tel: +82-2-880-1509, Fax: +82-2-888-1604

E-mail: byungskim@snu.ac.kr

hesion matrix. Therefore, it is necessary to further study the effects of GFs, cell-to-cell interaction, and biomaterials on chondrogenesis of MSCs.

GFs

Effect of GFs on chondrogenesis may differ depending on the GF dose, cell type and cell stage. Most researched GFs for chondrogenesis include transforming growth factor- β (TGF- β), bone morphogenic proteins (BMPs), insulin-like growth factor (IGF), and fibroblast growth factor (FGF). Stimulating MSCs with costly GFs usually requires high concentration and repeated treatments of GFs, and may cause side effects (2). Despite of these disadvantages, GFs are inevitably necessary for chondrogenesis of MSCs.

TGF- β

TGF- β superfamily includes TGF- β s, BMPs, activins and inhibins. The TGF- β family is associated with regulation of MSC proliferation, differentiation and ECM synthesis. TGF- β 1, - β 2, and - β 3 are secreted as an inactive form and are activated when they are separated from a latency-associated peptide (3). TGF- β attaches to type I and II receptor serine/threonine kinases and activates R-Smad proteins (4). R-Smad combines with Co-Smad, and then the activated complex is translocated into the nucleus, where it regulates gene expressions as a transcriptional factor (5).

TGF- β is an especially significant factor in chondrogenic differentiation of MSCs, and numerous researches have been reported on correlation between TGF- β and chondrogenesis (6). Large amount of latent TGF- β is already present in articular cartilage, and even tiny quantity of active TGF- β is considered to be a potent stimulator for proteoglycan and type II collagen synthesis (1). When properly treated with TGF- β , the number of chondrocytes differentiated from MSCs and their viability increase prominently (7). It is well documented that TGF- β induces expression of a transcriptional factor Sox9, an early gene of chondrogenesis, in its signaling pathway (8). Also, mRNA expression of collagen type II, an important marker of hyaline cartilage, is significantly enhanced by TGF- β . Aggrecan also shows similar tendency in presence of TGF- β . On the other hand, the expression of collagen I turns out to be much lower, showing another function of TGF- β to maintain hyaline cartilage phenotype of MSC-derived chondrocytes (9).

Controlled release system should be considered for an effective and long-lasting delivery of TGF- β . Continuous

treatment of TGF- β during chondrogenesis is not necessarily required, but it is critical at the first week *in vitro* (10). The delivery of TGF- β is more complicated in an *in vivo* environment than in an *in vitro* setting due to possible diffusions, immune responses, and proteolytic activities. TGF- β delivery usually requires a drug delivery scaffold, composed of biomaterials such as hyaluronic acid (HA), heparin, alginate and etc. In an experiment on nude mice, TGF- β was encapsulated in alginate microspheres, and they were laid in hyaluronic acid hydrogel with MSCs. When HA hydrogel was subcutaneously implanted on the nude mouse, TGF- β in microspheres was released for an extended time. Viability of MSCs remained high after a few weeks, and synthesis of collagen type II and aggrecan was prominently enhanced. However, after 8 weeks of implantation, calcification was observed, resulting in loss of lubricating hyaline phenotype. To alleviate such problem, co-delivery of parathyroid hormone-related protein (PTHrP) was used for its ability to reduce the calcium content in the region of implantation (7).

BMPs

BMP, generally known as cytokine, partly belongs to TGF- β family and plays an important role in forming bone and cartilage, inducing synergistic and overlapping effects each other (11). BMPs interact with cellular membrane receptors and trigger cascades in signal transduction through Smads, enhancing development of cartilage and bone (12). Mutations in BMP genes cause severe problems in skeletal development, such as murine brachypodism and human chondrodysplasia (11). Also, BMP deficient mice show low viability or severe appendicular skeletal defects (11).

Among them, BMP -2, -4, -6, -7, -13, and -14 are known to stimulate chondrogenesis of MSCs and induce specific gene expression for chondrogenic phenotype. Particularly, BMP-7 accelerates remodeling of chondrocytes and repair of full-thickness cartilage defects in the rabbits. The healing of full-thickness cartilage was also enhanced by combining BMP-7 and microfracture (13). BMP-2, 4, and 6 promote both transcription of collagen type II mRNA and differentiation of MSCs into chondrocytes (14). BMP-2 with Wnt-3A also enhances MSC chondrogenesis, while Wnt-7A induces dedifferentiation (15). BMP-2 can stimulate repair of lesions in cartilage in deeper hypoxic zones (16). Also, *ex vivo* retrovirally transduced stem cells with BMP-4 show enhanced chondrogenesis and improved repair in articular cartilage (17).

BMPs, implanted in ectopic localizations, may lead to terminal MSC differentiation into hypertrophy and sub-

sequent ossification (18). As a solution for reducing this problem, the Nogging delivery can be used to hinder the ossification triggered by BMP-4 (19). Therefore, proper injection site of BMPs and appropriate regulation of signaling pathway should be considered to improve efficacy in cartilage tissue engineering.

IGF

IGF, a protein with great sequence similarity to insulin, acts as the communication tool for the cells to interact with their environmental settings. IGF family includes two ligands IGF-1 and IGF-2, two cell-surface receptors IGF1R and IGF2R, six kinds of IGF-binding proteins (IGFBPs), and IGFBP proteases. IGF-1 and -2 are derived from insulin-like pre-propeptides and have a C-peptide bridge between the α - and β -chains in their molecular structures (20). IGF-1 binds to IGF1R and propagates its signal through the mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK) 1/2, and phosphatidylinositol (PI)-3-kinase-Akt pathways (21).

Generally, IGF-1 is associated with promoting biosynthetic and anabolic reactions. Natural mutations in IGF-1 gene, caused by the deletion of exons 4 and 5, result in severe retardation in skeletal development in both mice and human (22). Particularly in chondrogenesis, animal studies have shown that IGF-1 enhances synthesis of aggrecan, proteoglycans, and collagen type II (23). Study on the same model showed that the combined use of chondrocytes and IGF-1 not only formed collagen type II rich matrix, but also enhanced the overall continuity and consistency of the repaired tissues (1). IGF-1 and its receptor are expressed by chondrocytes, and they are the essential mediator of homeostasis in cartilage, promoting viability and proliferation of chondrocytes (24). With collagen type II matrix, cartilage defects filled with IGF-1 can be repaired more efficiently (25). IGF also shows synergistic effects with TGF- β , enhancing chondrogenesis of MSCs (26). While MSCs treated with TGF- β 3 alone produce both collagen type I and II, the production of collagen I in the MSCs treated with both TGF- β 3 and IGF-1 is minimal. BMP also has an effect similar to IGF-1 and minimizes the expression of collagen type I (27).

MSC-derived chondrocytes, however, may respond less to IGF-1 according to age- or osteoarthritis-associated factors. They seem to be partly related to over-expression of IGFBPs, since *in vivo* cartilage repair is dependent on the amount of IGFBPs produced. Also, the dose of IGF and its combination with other factors should be optimized for better results (1).

FGF

FGF includes 22 proteins in human and its molecular weight spans from 17kDa to 34kDa. FGFs are heparin-binding proteins and play an important role in the differentiation and proliferation of a wide range of cells (28). FGF interacts with one of the tyrosine kinase FGF receptors (FGFRs), activating PI3 kinase, Src, MAPKs, ERK, and p38 (29). Mutations of FGFRs usually lead to dysplasias, especially dwarfing chondrodysplasias. Also, FGF attaches to perlecan in pericellular matrix of cartilage, and acts as a mechanotransducer of chondrocytes (30).

FGF-2 is related to the proliferation and maintenance of multilineage differentiation capacity of MSCs. MSCs replicate more rapidly and differentiate into chondrocytes in medium with FGF-2 (1). Also, FGF-2 and FGF-18 together can regulate cartilage matrix homeostasis. Ca^{2+} ions are involved in a signaling pathway leading to the expression of FGF-18, which promotes differentiation of MSCs while suppressing their proliferation. It was also discovered that IGF-1 and FGF-2 together increase cartilage repair in a xenogenic transplant (31).

However, FGF-2 can block the synergistic effect between BMP-2 and sonic-hedgehog-transfected progenitors of chondrocytes (32). Also, co-treatment of FGF-2 with BMP-6 offsets the chondrogenic capacity of BMP-6 (33). FGF-2 may inhibit TGF- β , hence weakening induction of chondrogenesis in murine MSCs (34). In addition, FGFR-2, one of the early genes up-regulated during limb development, negatively regulates chondrogenesis and proliferation of MSCs through MAPK pathway and STAT1 pathway, relatively (35).

Cell-to-cell interaction

Cellular interaction is one of the key factors for successful chondrogenesis of MSCs. Strong cellular interaction mediated by cell adhesion molecules enables MSCs to differentiate into prechondroblasts during limb development (36). For cell-to-cell interaction, maintenance of appropriate cell density is crucial; 5×10^7 MSCs/ml embedded in collagen type I gel showed chondrogenic phenotype. On the other hand, cells of less than 1×10^6 MSCs/ml failed in chondrogenesis due to poor cell proliferation and increased apoptosis (37).

Three-dimensional (3D) culture promotes chondrogenesis of MSCs due to increased possibility of cellular interactions. Different from monolayer culture of the MSCs, 3D culture mimics the microenvironmental settings of the body, providing a similar environment for the

MSCs *in vivo*. For enhanced 3D cellular interaction, MSCs should be cultured in pellet rather than in monolayer. MSCs in pellet culture show superior chondrocytic phenotype than those in monolayer culture during chondrogenesis (36). Culture of pellets, which can be obtained from centrifugation of the cells, offers favorable 3D structure for cells, enabling cell-to-cell interactions. Direct cellular interactions enhance the expression of specific genes, thus determining cartilage phenotype and cell proliferation state. Although the mechanism of interaction among the cells is not yet known, cross talk via gap junctions may be a suggestion for that (38).

Quantitative real time-PCR (qRT-PCR) indicates an increase of Sox9 mRNA in 3D cultured MSCs compared to monolayer cultured MSCs. Sox9, a member of the Sry-type HMG box gene family, has been shown to activate collagen type II and aggrecan. After the increase in Sox9 mRNA expression, the production of collagen type II, aggrecan, and cartilage oligomeric matrix protein (COMP) is observed (3). Also, when treated with TGF- β , MSCs in monolayer show increased yet limited chondrogenic gene expression. However, pellet-cultured MSCs with TGF- β express considerably higher amount of chondrogenic genes, and unlike MSCs in monolayer where the collagen produced changes from type II to type I, 3D cultured MSCs synthesize greater amount collagen type II (3).

Co-culture model induces cell-to-cell contacts, modulating the phenotype of the cells. Strong correlation between gene expression and cellular interaction has been studied (38). Chondrocytes and osteoblasts can mutually influence themselves when they are co-cultured in direct contact with each other. In the co-culture system, chondrocytes synthesized more collagen type II and less glycosaminoglycans (GAGs). Inversely, osteoblasts directly seeded onto chondrocytes showed slow mineralization and high production rate of collagen type I (39).

MSC differentiation in co-culture depends on the type of co-cultured cells, such as chondrocytes, nucleus pulposus (NP) cells, and annulus fibrosis (AF) cells. First, MSCs co-cultured with cartilage tissue and chondrocytes produce more Sox9 and collagen type II. Co-cultured chondrocytes attributes to the high density of newly synthesized cartilage matrix, in compensation for supplementation of GFs. These changes can be made solely by paracrine contacts without direct contacts between the cells (38). IGF-1, which is expressed by co-cultured chondrocytes, may be responsible for promoting chondrogenesis of MSCs (1). Next, co-cultured NP cells influence MSCs by enhancing the expression of Sox9, collagen type II, and aggrecan only after 7 days of co-culture. In an ex-

periment, the best result was observed when the cell number ratio of NP cells to MSCs was 3 to 1 (39). Also, co-culture of AF cells and MSCs resulted in augmented production of GAGs (40).

Finally, MSCs co-cultured with CD45-positive cells, show an enhanced expression of specific genes, the markers of chondrogenic phenotype (41). CD45, also known as protein tyrosine phosphatase receptor type C, a type I transmembrane protein, is expressed in differentiated hematopoietic cells (42). In bone marrow, MSCs and hematopoietic stem cells co-exist and can be discerned with the presence of CD45. CD45 seems to transfer intercellular signals between hematopoietic cells and MSCs. The co-culture system with CD45-positive hematopoietic cells offers favorable microenvironment for MSCs to induce a transcriptional factor Sox9, resulting in a considerable increase in the expression of collagen type II, COMP, and aggrecan. Genes related to osteogenesis are also up-regulated in MSCs co-cultured with CD45-positive cells, expressing more collagen type I and type X, the marker molecules of hypertrophy. Thus, CD45-positive cells are crucial in progressing neighboring MSCs into pre-hypertrophic and finally hypertrophic stage, frustrating sustainable hyaline cartilage production (43).

Biomaterials

Biomaterials are required during chondrogenesis of MSCs for several reasons. They offer cell adhesion sites and allow diffusion of nutrients, oxygen and cells. Thus, their ideal properties depend on their porosity and 3D shape (1). Also, to avoid inflammatory reactions, injected biomaterials should be biocompatible with the host body. Biomaterials also serve as a scaffold that can mechanically support lesions in articular cartilage. In addition, homogeneous and bio-active biomaterials can be used for successful delivery and uniform release of growth factors. Injected bio-scaffolds need to adhere to the host matrix and bio-degrade gradually after implantation.

HA is a useful natural matrix that provides a stable three-dimensional environment and induces chondrogenesis of MSCs (43). HA is biocompatible when cross-linked into hydrogel form, which can be less invasively injected and solidified (44). HA acts as a physical stabilizer of the formed hydrogel, and can communicate with MSCs via cell surface receptors. The process of crosslinking is controlled by pH, temperature, ionic environment, and ultraviolet rays (45). HA hydrogel also increases the synthesis of ECM of chondrocytes and captures high portions of water, which facilitates rapid diffusion of the cells and

nutrients (45). Also, growth factors can be incorporated into and delivered uniformly through this naturally biodegrading HA hydrogels (45). Furthermore, HA itself is associated with up-regulation of collagen type II in comparison to poly (ethylene glycol) (PEG). In the photo-cross-linked HA hydrogel, both the expression of Sox9 and the synthesis of cartilage matrix proteins of MSCs are higher than in PEG gel (43).

Alginate, a natural molecule, can be shaped as a microsphere in order to elongate the releasing time of growth factors, hence providing a controlled release of growth factors both *in vitro* and *in vivo*. In an experiment, TGF- β was encapsulated in alginate microspheres coated with nano particles that were fabricated to reduce the initial burst of GFs. Then, the microspheres and human MSCs were seeded into HA hydrogel. By controlling the release of TGF- β , MSCs with TGF- β encapsulated microspheres produced much more collagen type II and chondroitin sulfate but less collagen type I, showing better chondrogenic differentiation than the no-microsphere control group. In addition, prolonged release time of over three days strengthened the mechanical strength of newly synthesized cartilage tissues (7).

Collagen type II, a major component in hyaline cartilage, has many advantages as a biomaterial for chondrogenesis, such as biodegradability and its ability to induce repair processes in articular cartilage (45). MSCs in collagen hydrogels continuously increase the expression of collagen type II and aggrecan mRNAs than in other biomaterials. This effectiveness of chondrogenesis induction can be measured by qRT-PCR and *in situ* hybridization. Co-treatment of collagen type II matrix with TGF- β 1 and dexamethasone makes a more favorable environment for chondrogenic differentiation.

Synthetic or hybrid materials can also be good candidates for scaffolds in MSC chondrogenesis for their high resistance to mechanical pressures, which makes them more useful in clinical applications compared to mechanically fragile natural materials. Also, synthetic materials can be fabricated more uniformly and purely. Polyglycolic acid, polylactides, PEG, and poly(L-lactide- ϵ -caprolactone) have been studied as synthetic scaffolds. Poly (L-lactide- ϵ -caprolactone) can be produced as a long standing structure similar to the shape of natural cartilage (46). Next, hybrid scaffolds are comprised of both natural and synthetic materials. They show superior characteristics, combining advantages from each side; biocompatibility of natural materials and mechanical strength of synthetic materials. As an example, polylactide/ alginate amalgam has been known to support the chondrogenesis of human

MSCs *in vitro* (47). Another hybrid scaffold, composed of Poly (N-isopropylacrylamide) (PNIPAAm) and water soluble chitosan (WSC), has been also investigated for its water-solubility and low critical temperature (32°C) (48). This thermosensitive WSC-g- PNIPAAm gel can be injected noninvasively and enhance the expression of aggrecan and collagen II (49).

Summary

As a solution for the low regenerative capacity of articular cartilage, adult MSC has attracted many researchers' attention for its self-renewable and pliable traits. By using MSCs, donor site morbidity and immune reactions can be overcome. However, due to multilineage differentiation capacity of MSCs, proper stimulations, such as GFs, cellular contacts, and biomaterials should be given for successful chondrogenesis. Several kinds of GFs are associated with various signaling pathways in MSCs, and each of them induces the expression of specific genes involved in chondrogenesis. Also, as cell-to-cell interaction is crucial for chondrogenic differentiation, MSCs in pellet culture can show superior chondrocytic phenotype. The phenotype of differentiated MSCs may differ according to the type of co-cultured cells. Finally, biocompatible materials, such as HA, alginate, and collagen type II in particular, serve as scaffolds for adhesion of MSCs and as the delivery tools of GFs, consequently stimulating chondrogenesis. However, the composite effects of these various factors have not yet been thoroughly investigated, and ossification or progression into hypertrophy should be controlled for clinical applications. Nevertheless, research on MSCs and tissue engineering is progressing rapidly, and MSCs are expected to be generally applied for cartilage repair in the future.

Acknowledgments

This study was supported by a grant (A100443) from the Korean Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea.

Potential conflict of interest

The authors have no conflicting financial interest.

References

1. Vinatier C, Bouffi C, Merceron C, Gordeladze J, Brondello JM, Jorgensen C, Weiss P, Guicheux J, Noël D. Cartilage tissue engineering: towards a biomaterial-assisted mesenchymal stem cell therapy. *Curr Stem Cell Res Ther* 2009;4:318-329

2. Koga H, Engebretsen L, Brinchmann JE, Muneta T, Sekiya I. Mesenchymal stem cell-based therapy for cartilage repair: a review. *Knee Surg Sports Traumatol Arthrosc* 2009;17:1289-1297
3. Bosnakovski D, Mizuno M, Kim G, Takagi S, Okumura M, Fujinaga T. Chondrogenic differentiation of bovine bone marrow mesenchymal stem cells (MSCs) in different hydrogels: influence of collagen type II extracellular matrix on MSC chondrogenesis. *Biotechnol Bioeng* 2006;93:1152-1163
4. Lebrin F, Deckers M, Bertolino P, Ten Dijke P. TGF-beta receptor function in the endothelium. *Cardiovasc Res* 2005;65:599-608
5. Yue J, Mulder KM. Transforming growth factor-beta signal transduction in epithelial cells. *Pharmacol Ther* 2001;91:1-34
6. Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU. *In vitro* chondrogenesis of bone marrow-derived mesenchymal progenitor cells. *Exp Cell Res* 1998;238:265-272
7. Bian L, Zhai DY, Tous E, Rai R, Mauck RL, Burdick JA. Enhanced MSC chondrogenesis following delivery of TGF- β 3 from alginate microspheres within hyaluronic acid hydrogels *in vitro* and *in vivo*. *Biomaterials* 2011;32:6425-6434
8. Kawakami Y, Rodriguez-León J, Izpisua Belmonte JC. The role of TGFbetas and Sox9 during limb chondrogenesis. *Curr Opin Cell Biol* 2006;18:723-729
9. Roman-Blas JA, Stokes DG, Jimenez SA. Modulation of TGF-beta signaling by proinflammatory cytokines in articular chondrocytes. *Osteoarthritis Cartilage* 2007;15:1367-1377
10. Buxton AN, Bahney CS, Yoo JU, Johnstone B. Temporal exposure to chondrogenic factors modulates human mesenchymal stem cell chondrogenesis in hydrogels. *Tissue Eng Part A* 2011;17:371-380
11. Chen D, Zhao M, Mundy GR. Bone morphogenetic proteins. *Growth Factors* 2004;22:233-241
12. Bleuming SA, He XC, Kodach LL, Hardwick JC, Koopman FA, Ten Kate FJ, van Deventer SJ, Hommes DW, Peppelenbosch MP, Offerhaus GJ, Li L, van den Brink GR. Bone morphogenetic protein signaling suppresses tumorigenesis at gastric epithelial transition zones in mice. *Cancer Res* 2007;67:8149-8155
13. Kuo AC, Rodrigo JJ, Reddi AH, Curtiss S, Grotkopp E, Chiu M. Microfracture and bone morphogenetic protein 7 (BMP-7) synergistically stimulate articular cartilage repair. *Osteoarthritis Cartilage* 2006;14:1126-1135
14. Weber M, Steinert A, Jork A, Dimmler A, Thürmer F, Schütze N, Hendrich C, Zimmerman U. Formation of cartilage matrix proteins by BMP-transfected murine mesenchymal stem cells encapsulated in a novel class of alginates. *Biomaterials* 2002;23:2003-2013
15. Fischer L, Boland G, Tuan RS. Wnt-3A enhances bone morphogenetic protein-2-mediated chondrogenesis of murine C3H10T1/2 mesenchymal cells. *J Biol Chem* 2002;277:30870-30878
16. Gelse K, Mühle C, Franke O, Park J, Jehle M, Durst K, Göken M, Hennig F, von der Mark K, Schneider H. Cell-based resurfacing of large cartilage defects: long-term evaluation of grafts from autologous transgene-activated periosteal cells in a porcine model of osteoarthritis. *Arthritis Rheum* 2008;58:475-488
17. Kuroda R, Usas A, Kubo S, Corsi K, Peng H, Rose T, Cummins J, Fu FH, Huard J. Cartilage repair using bone morphogenetic protein 4 and muscle-derived stem cells. *Arthritis Rheum* 2006;54:433-442
18. Noël D, Gazit D, Bouquet C, Apparailly F, Bony C, Plence P, Millet V, Turgeman G, Perricaudet M, Sany J, Jorgensen C. Short-term BMP-2 expression is sufficient for *in vivo* osteochondral differentiation of mesenchymal stem cells. *Stem Cells* 2004;22:74-85
19. Hannallah D, Peng H, Young B, Usas A, Gearhart B, Huard J. Retroviral delivery of Noggin inhibits the formation of heterotopic ossification induced by BMP-4, demineralized bone matrix, and trauma in an animal model. *J Bone Joint Surg Am* 2004;86-A:80-91
20. Lehmann M, André F, Bellan C, Remacle-Bonnet M, Garrouste F, Parat F, Lissitsky JC, Marvaldi J, Pommier G. Deficient processing and activity of type I insulin-like growth factor receptor in the furin-deficient LoVo-C5 cells. *Endocrinology* 1998;139:3763-3771
21. Ross RJ, Rodriguez-Arnan J, Benthall J, Coakley JH. The role of insulin, growth hormone and IGF-I as anabolic agents in the critically ill. *Intensive Care Med* 1993;19 Suppl 2:S54-57
22. Woods KA, Camacho-Hübner C, Savage MO, Clark AJ. Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. *N Engl J Med* 1996;335:1363-1367
23. Martin JA, Scherb MB, Lembke LA, Buckwalter JA. Damage control mechanisms in articular cartilage: the role of the insulin-like growth factor I axis. *Iowa Orthop J* 2000;20:1-10
24. Yoon DM, Fisher JP. Effects of exogenous IGF-1 delivery on the early expression of IGF-1 signaling molecules by alginate embedded chondrocytes. *Tissue Eng Part A* 2008;14:1263-1273
25. Schmidt MB, Chen EH, Lynch SE. A review of the effects of insulin-like growth factor and platelet derived growth factor on *in vivo* cartilage healing and repair. *Osteoarthritis Cartilage* 2006;14:403-412
26. Fukumoto T, Sperling JW, Sanyal A, Fitzsimmons JS, Reinholz GG, Conover CA, O'Driscoll SW. Combined effects of insulin-like growth factor-I and transforming growth factor-beta1 on periosteal mesenchymal cells during chondrogenesis *in vitro*. *Osteoarthritis Cartilage* 2003;11:55-64
27. Indrawattana N, Chen G, Tadokoro M, Shann LH, Ohgushi H, Tateishi T, Tanaka J, Bunyaratvej A. Growth factor combination for chondrogenic induction from human mesenchymal stem cell. *Biochem Biophys Res Commun* 2004;320:914-919
28. Ornitz DM. FGFs, heparan sulfate and FGFRs: complex interactions essential for development. *Bioessays* 2000;22:108-112

29. Shanmugaraian TS, Kim BS, Lee HS, Im GI. Growth factors and signaling pathways in the chondrogenic differentiation of mesenchymal stem cells. *Tissue Eng Regen Med* 2011;8:292-299
30. Vincent TL, McLean CJ, Full LE, Peston D, Saklatvala J. FGF-2 is bound to perlecan in the pericellular matrix of articular cartilage, where it acts as a chondrocyte mechanotransducer. *Osteoarthritis Cartilage* 2007;15:752-763
31. Madry H, Orth P, Kaul G, Zurakowski D, Menger MD, Kohn D, Cucchiariini M. Acceleration of articular cartilage repair by combined gene transfer of human insulin-like growth factor I and fibroblast growth factor-2 in vivo. *Arch Orthop Trauma Surg* 2010;130:1311-1322
32. Enomoto-Iwamoto M, Nakamura T, Aikawa T, Higuchi Y, Yuasa T, Yamaguchi A, Nohno T, Noji S, Matsuya T, Kurisu K, Koyama E, Pacifici M, Iwamoto M. Hedgehog proteins stimulate chondrogenic cell differentiation and cartilage formation. *J Bone Miner Res* 2000;15:1659-1668
33. Hildner F, Peterbauer A, Wolbank S, Nürnberger S, Marlovits S, Redl H, van Griensven M, Gabriel C. FGF-2 abolishes the chondrogenic effect of combined BMP-6 and TGF-beta in human adipose derived stem cells. *J Biomed Mater Res A* 2010;94:978-987
34. Baddoo M, Hill K, Wilkinson R, Gaupp D, Hughes C, Kopen GC, Phinney DG. Characterization of mesenchymal stem cells isolated from murine bone marrow by negative selection. *J Cell Biochem* 2003;89:1235-1249
35. Liu Z, Xu J, Colvin JS, Ornitz DM. Coordination of chondrogenesis and osteogenesis by fibroblast growth factor 18. *Genes Dev* 2002;16:859-869
36. Haas AR, Tuan RS. Chondrogenic differentiation of murine C3H10T1/2 multipotential mesenchymal cells: II. Stimulation by bone morphogenetic protein-2 requires modulation of N-cadherin expression and function. *Differentiation* 1999;64:77-89
37. Koga H, Muneta T, Nagase T, Nimura A, Ju YJ, Mochizuki T, Sekiya I. Comparison of mesenchymal tissues-derived stem cells for in vivo chondrogenesis: suitable conditions for cell therapy of cartilage defects in rabbit. *Cell Tissue Res* 2008;333:207-215
38. Grassel S, Ahmed N. Influence of cellular microenvironment and paracrine signals on chondrogenic differentiation. *Front Biosci* 2007;12:4946-4956
39. Jiang J, Nicoll SB, Lu HH. Co-culture of osteoblasts and chondrocytes modulates cellular differentiation *in vitro*. *Biochem Biophys Res Commun* 2005;338:762-770
40. Le Visage C, Kim SW, Tateno K, Sieber AN, Kostuik JP, Leong KW. Interaction of human mesenchymal stem cells with disc cells: changes in extracellular matrix biosynthesis. *Spine (Phila Pa 1976)* 2006;31:2036-2042
41. Ahmed N, Vogel B, Rohde E, Strunk D, Grifka J, Schulz MB, Grassel S. CD45-positive cells of haematopoietic origin enhance chondrogenic marker gene expression in rat marrow stromal cells. *Int J Mol Med* 2006;18:233-240
42. Im M, Chae H, Kim T, Park HH, Lim J, Oh EJ, Kim Y, Park YJ, Han K. Comparative quantitative analysis of cluster of differentiation 45 antigen expression on lymphocyte subsets. *Korean J Lab Med* 2011;31:148-153
43. Chung C, Burdick JA. Influence of three-dimensional hyaluronic acid microenvironments on mesenchymal stem cell chondrogenesis. *Tissue Eng Part A* 2009;15:243-254
44. Nesic D, Whiteside R, Brittberg M, Wendt D, Martin I, Mainil-Varlet P. Cartilage tissue engineering for degenerative joint disease. *Adv Drug Deliv Rev* 2006;58:300-322
45. Drury JL, Mooney DJ. Hydrogels for tissue engineering: scaffold design variables and applications. *Biomaterials* 2003;24:4337-4351
46. Li WJ, Tuli R, Okafor C, Derfoul A, Danielson KG, Hall DJ, Tuan RS. A three-dimensional nanofibrous scaffold for cartilage tissue engineering using human mesenchymal stem cells. *Biomaterials* 2005;26:599-609
47. Caterson EJ, Nesti LJ, Li WJ, Danielson KG, Albert TJ, Vaccaro AR, Tuan RS. Three-dimensional cartilage formation by bone marrow-derived cells seeded in polylactide/alginate amalgam. *J Biomed Mater Res* 2001;57:394-403
48. Cho JH, Kim SH, Park KD, Jung MC, Yang WI, Han SW, Noh JY, Lee JW. Chondrogenic differentiation of human mesenchymal stem cells using a thermosensitive poly (N-isopropylacrylamide) and water-soluble chitosan copolymer. *Biomaterials* 2004;25:5743-5751
49. Lee JW, Kim YH, Kim SH, Han SH, Hahn SB. Chondrogenic differentiation of mesenchymal stem cells and its clinical applications. *Yonsei Med J* 2004;45 Suppl:41-47