

Review Article

Cytokine networking of chondrocyte dedifferentiation *in vitro* and its implications for cell-based cartilage therapy

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Abstract: Autologous chondrocyte implantation (ACI) is a golden treatment for large defects of the knee joint without osteoarthritis or other complications. Despite notable progresses, generation of a stable chondrocyte phenotype using progenitor cells remains a main obstacle for chondrocyte-based cartilage treatment. Monolayer chondrocyte expansion *in vitro* is accompanied by chondrocyte dedifferentiation, which produces a non-specific mechanically inferior extracellular matrix (ECM) unsuitable for ACI. In-depth understanding of the molecular events during chondrocyte dedifferentiation is required to maintain the capacity of *in vitro* expanded chondrocytes to produce hyaline cartilage-specific ECM. This review discusses key cytokines and signaling pathways involved in chondrocyte dedifferentiation from the standpoint of catabolism and anabolism. Some potential therapeutic strategies are also presented to counteract chondrocyte dedifferentiation for cell-based cartilage therapy.

Keywords: Chondrocyte dedifferentiation, *in vitro*, cytokines, signaling pathways, cartilage repair, autologous chondrocyte implantation

Introduction

Articular cartilage is an avascular and aneural load-bearing connective tissue with a very limited capacity for intrinsic repair. Once damaged by trauma or pathology, it is highly susceptible to structural degradation, making it particularly difficult to restore. The chondrocyte is the only cell type that resides within articular cartilage and is solely responsible for the synthesis and turnover of the extracellular matrix (ECM).

For more than decades, autologous chondrocyte implantation (ACI) has been the golden therapy for patients with focal lesions of articular cartilage [1]. ACI procedure consists of three stages (**Figure 1**). The first stage is to harvest around 100 milligrams (mg) cartilage from a non-weight bearing area of the patient under arthroscopy. The chondrocytes are enzymatically isolated from cartilage and propagated to obtain enough cells *in vitro* in a specialized laboratory. These cells are re-implanted in com-

ination with a membrane (tibial periosteum or biomembrane) in the damaged area of the articular cartilage when the patient undergoes a second treatment. ACI treatment leads to satisfying clinical results in terms of patient satisfaction, reduction of pain and improvement in knee function [2]. Nevertheless, it is not yet a routine application in clinical practice, with its application being mainly limited to young patients. One of the technical challenges is the isolation and *ex vivo* expansion of a large enough number of differentiated articular chondrocytes. During this process, the chondrocytes become fibroblast-like cells (**Figure 2**), characterized by an increase in the expression of type I collagen (COL-1) and a decrease of cartilage-specific markers, such as type II, XI, IX collagens (COL-2, COL-11, COL-9) and aggrecan (ACAN) [3-5]. This process is designated as chondrocyte dedifferentiation. The dedifferentiated chondrocytes produce a non-specific mechanically inferior ECM and are no longer suitable for ACI. Therefore, chondrocyte dedifferentiation is a major

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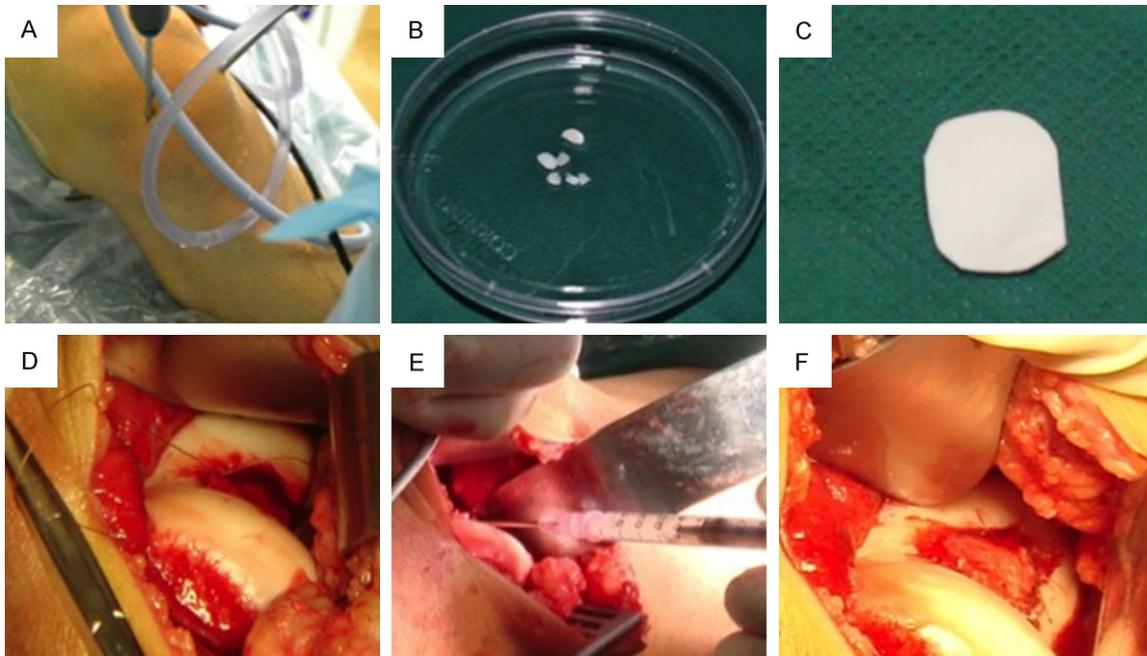


Figure 1. Autologous chondrocyte implantation. A. Cartilage biopsy was harvested from a non-weight bearing area of the knee joint under an arthroscope. B. Around 100 mg cartilage was harvested and enzymatically digested. Chondrocytes were propagated in a GMP lab. C. During the second operation, a collagen membrane was trimmed according to the defect size. D. The periosteum was sutured. E. Chondrocytes were injected into the space between the membrane and the defect. F. After chondrocyte injection, the membrane was sealed with fibrin glue to prevent leaking.

obstacle in cell-based cartilage repair. In the past decades, multiple studies have been carried out to explore the molecular mechanisms underlying chondrocyte dedifferentiation.

This review focuses on cytokines and signaling pathways of the anabolism and catabolism (summarized in **Table 1**), which are involved in chondrocyte phenotype maintenance. The purpose of this report is to provide an up-to-date overview of cytokine networking involved in chondrocyte dedifferentiation *in vitro* and to provide potential targets for improving autologous chondrocyte-based cartilage repair.

Imbalance between catabolism and anabolism

The major function of chondrocytes is to produce cartilage ECM. In turn, the ECM modifies chondrocyte behavior. Metabolic balance of ECM could play a critical role in chondrocyte phenotype maintenance. The failure to maintain the anabolic-catabolic balance within the cartilage matrix may give rise to chondrocyte dedifferentiation. The catabolic genes, such as inflammatory cytokines and proteases, partici-

pate in cartilage matrix degradation. In contrast, the anabolic genes including growth factors (e.g. insulin-like growth factor, bone morphogenetic protein) critically contribute to cartilage matrix production and maintenance.

Inflammatory cytokines

Interleukin 1-beta (IL-1 β), the major pro-inflammatory cytokine in osteoarthritic synovial fluid, induces the dedifferentiation of cultured articular chondrocytes by decreasing COL-2 while increasing COL-1 and COL-3 expression at mRNA and protein levels [6, 7]. During chondrocyte dedifferentiation, IL-1 β up-regulates the synthesis of E-series prostaglandins (PGE) to induce chondrocyte differentiation and increase COL-2 expression, thereby counteracting the dedifferentiation-promoting action of IL-1 β [8, 9]. Thus, the role of IL-1 β on chondrocytes appears to be paradoxical. On one hand, IL-1 β directly promotes chondrocyte dedifferentiation; on the other hand, it maintains chondrocytes in differentiated status through increasing PGE. To further understand this paradox, several downstream signal molecules have been studied in IL-1 β -treated articular chondro-

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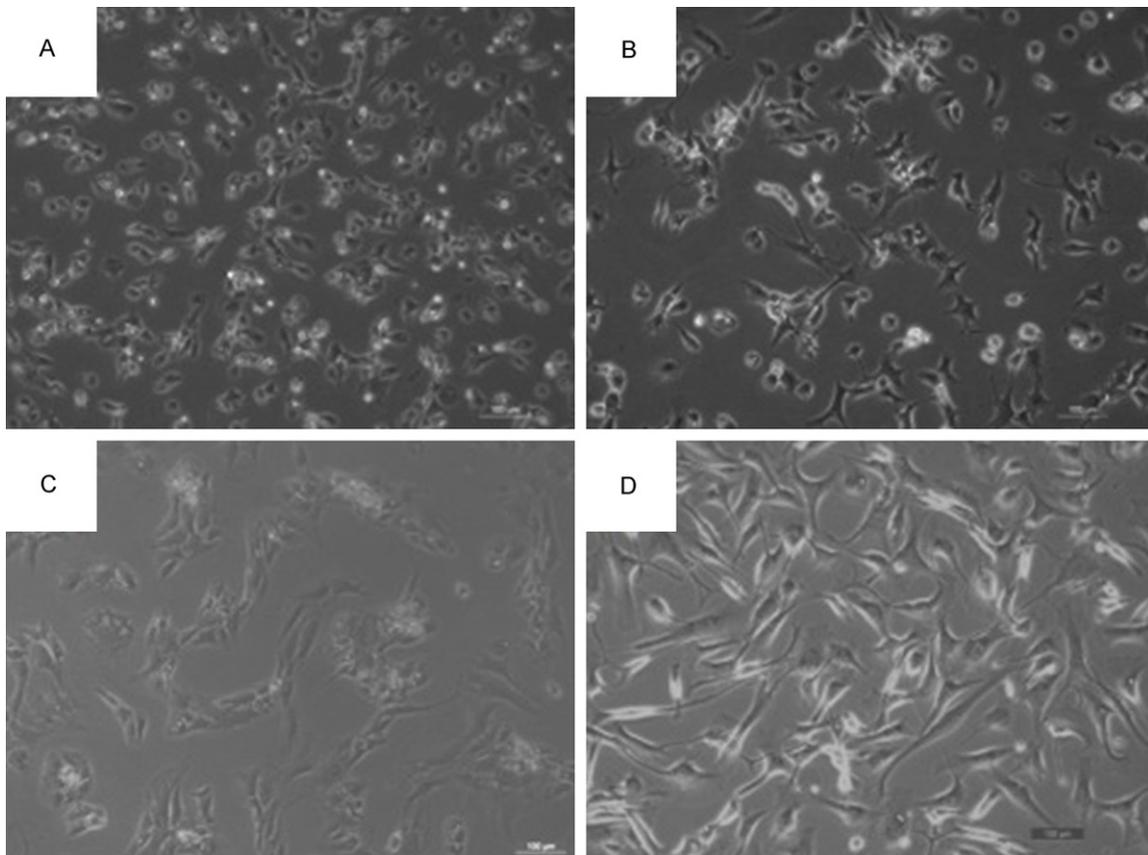


Figure 2. Chondrocyte dedifferentiation during *in vitro* expansion. Primary human chondrocyte morphology under invert microscope ($\times 100$). With passaging, round and polygonal chondrocytes were shifted to fibroblast-like cells. A (P0), B (P1), C (P2), D (P4).

cytes and found that IL-1 β activates c-Jun-N-terminal kinase (c-JNK), p38 mitogen-activated protein kinase (MAPK) and extracellular-signal-regulated protein kinase (ERK) -1/2 in primary cultures of human articular chondrocytes [10]. ERK-1/2 is the major tyrosine-phosphorylated protein in IL-1 β stimulated chondrocytes. In response to IL-1 β stimulation, ERK and p38 MAPK are activated at 5 minutes and JNK is activated at 15 minutes [11]. Interestingly, prolonged IL-1 β stimulation for 24 hours results in ERK-1/2 inhibition in chondrocytes [12]. Thus, the role of ERK-1/2 in chondrocyte dedifferentiation remains controversial. The dedifferentiated chondrocytes express lower levels of cyclooxygenase-2 (COX-2) mRNA and synthesize less PGE₂ than differentiated chondrocytes. The down-regulation of COX-2 expression is associated with a reduction in IL-1 β -induced p38 MAPK activation [13]. The WNT family protein (WNT-3A, WNT-7A) activities are also enhanced during IL-1 β -induced dedifferentiation of articular chondrocytes [14, 15].

Treating lapine articular chondrocytes with human recombinant IL-1 β has been shown to boost the production of nitric oxide (NO) and reduce COL-2 synthesis. NO activates ERK-1/2 and p38 MAPK, but inhibits protein kinase C alpha (PKC- α) and PKC zeta (PKC- ζ) in chondrocytes [16]. NO appears to affect the metabolism of cartilage in a stimulus-dependent manner, as NO has been shown to enhance tumor necrosis factor (TNF)- α -induced, but not IL-1 β -induced, ACAN degradation in bovine cartilage explants [17]. Taken together, IL-1 β regulates chondrocyte dedifferentiation through multiple pathways and the balance between the activities of these pathways may determine the overall effect of IL-1 β .

In addition to IL-1 β , the role of IL-6 in chondrocyte dedifferentiation has attracted much attention. IL-6 is produced by osteoarthritic synovial lining or expanded chondrocytes rather than by cartilage [18], which exerts catabolic effects on cartilage and chondrocytes [19, 20].

Cytokines in chondrocyte dedifferentiation

Table 1. Summary of cytokines and signaling pathways involved in chondrocyte dedifferentiation

Cytokine	Signaling pathway	Effect on chondrocyte phenotype	Reference
IL-1 β	p38MAPK, ERK, JNK, PKC α , PKC ζ , WNT-3A, WNT-7A	(-) COL-2; (+) COL-1; (+) COL-3	[6, 7, 10-12, 14-16]
IL-6	Notch	(-) ACAN; (+) MMP-13	[18-21]
IGF-I	PI3K/AKT, ERK/MAPK	(+) ACAN; (+) COL-2; (+) COL-1; (+) COL-10	[33-41]
PDGF	ERK, PI3K/AKT	undefined	[20, 42-44]
TGF- β 1	ERK/AP-1, SP3/SP1	(\pm) COL-2* (+) COL-1	[45-52]
BMP-2	p38MAPK, WNT-7A	(+) COL-2 (+) ACAN (+) COL-1	[38, 62-68]
FGF-18	ERK, p38MAPK	undefined	[72-74]

*Species - dependent: rabbit/goat/mouse: (+) COL-2; human: (-) COL-2, (-) ACAN. (+) indicates up-regulation; (-) indicates down-regulation.

According to a recent report, IL-6 induces similar effects as Notch activation in chondrocytes. Furthermore, IL-6 mediates the effects of Notch signaling pathway through ACAN suppression and MMP-13 induction [21].

Proteases

Cartilage matrix components are degraded by various proteases, such as cathepsins [22], matrix metalloproteinases (MMPs) [23] and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) [24]. A microarray analysis revealed that 84 genes are up-regulated and 56 genes are down-regulated in passage 4 (P4) human chondrocytes compared to passage 1 (P1) cells. Among them, cathepsin K (CTSK) expression level is increased 28-fold. Overexpression of CTSK leads to reduced matrix production in cultured human chondrocytes *in vitro* and poor formation of engineered cartilage *in vivo*. In contrast, CTSK knockdown increases the capability to maintain the chondrogenic phenotype of *in vitro* expanded cells with increased mRNA and protein expression of COL-2 and ACAN, in comparison to control cells [25].

To date, the role of MMP-13 in COL-2 degradation [26, 27] has been established, which critically contributes to the pathogenesis of experi-

mental osteoarthritis (OA) [28] and human OA [29]. However, there are conflicting results about the expression changes of MMP-13 during chondrocyte expansion. Two studies found that the expression of MMP-13 decreases during cell passaging [30, 31] while another *in vitro* study reported that MMP-13 expression is increased from day 1 up to 21 [32]. Moreover, chitosan-plasmid DNA nanoparticles encoding short hairpin RNA (shRNA) against MMP-13 has been shown to inhibit the expression of dedifferentiation-related genes and help regenerating durable cartilage [33]. Therefore, genetic modification of MMP-13 or CTSK could be a promising strategy to block chondrocyte dedifferentiation from the standpoint of catabolism.

Insulin-like growth factor-I

Insulin-like growth factor-I (IGF-I), one of the most important anabolic growth factors for articular cartilage, plays a key role in cartilage homeostasis, balancing proteoglycans synthesis and breakdown [34]. Several studies have demonstrated that IGF-I is not only able to extend the chondrogenic potential of dedifferentiated chondrocytes *in vitro* [35], but also can enhance the chondrogenesis of bovine articular chondrocytes seeded into polymer scaffolds [36]. Interaction of IGF-I and IGF-1R activates both the phosphoinositide-3 kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) and ERK-MAPK pathways, but only the activation of the PI3K/AKT/mTOR pathway is responsible for the ability of IGF-I to increase proteoglycan synthesis of adult human articular chondrocytes [37, 38]. The transfection of IGF-I gene (tIGF-I) into chondrocytes stimulates both COL-2 and ACAN expression. Of note, in comparison to the individual transgenes of BMP-2, BMP-7, TGF- β 1 and FGF-2, tIGF-I generates the maximal stimulation of COL-2 expression in bovine articular chondrocytes [39].

Monolayer chondrocytes rapidly lose their differentiated phenotype, which is accompanied by a significant decrease in IGF-I expression [30]. IGF-I drives dedifferentiated chondrocytes

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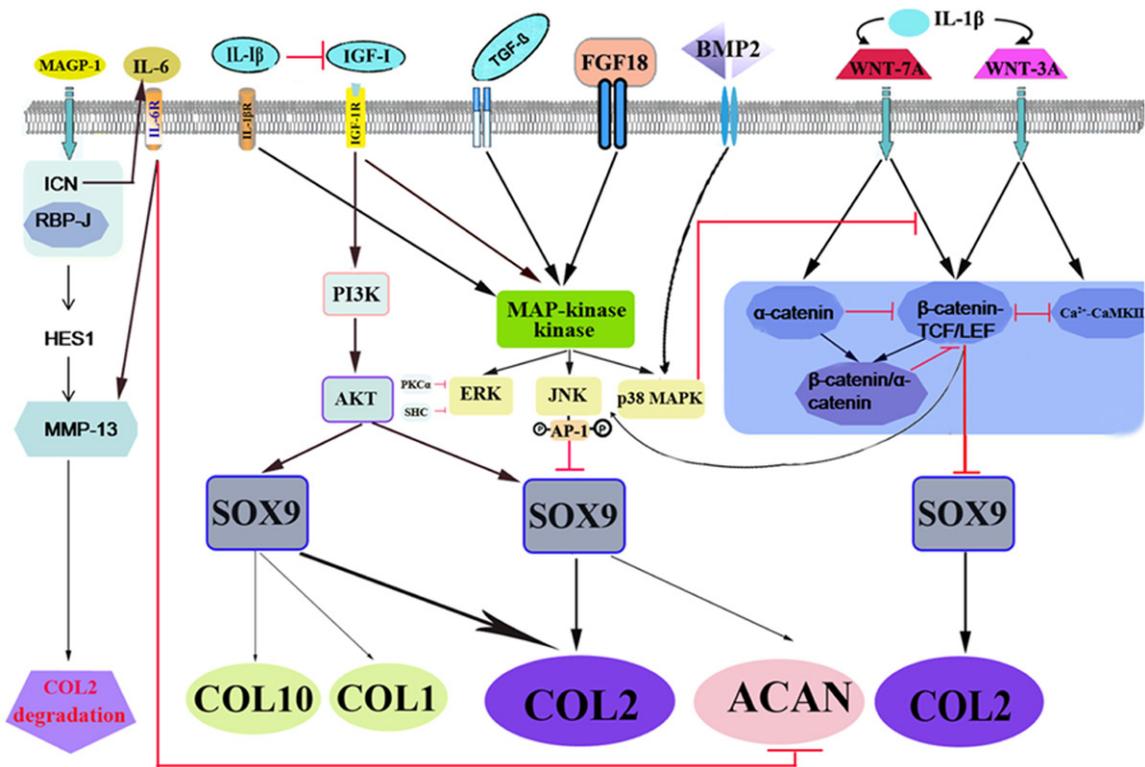


Figure 3. Schematic illustration of molecular events in chondrocyte dedifferentiation. Multiple signaling pathways are evoked during chondrocyte undergoing stimulants, including catabolic factors (IL-1 β , IL-6, FGF-18) and anabolic factors (IGF-1, BMP-2, TGF- β). MAPK, PI3K/AKT, PKC, WNT, and Notch proteins are involved in regulating the ratio of COL-2 and COL-1, a key indicator of chondrocyte dedifferentiation. The red dash indicates inhibition and the black arrow indicates promotion.

re-differentiation back to the differentiated phenotype. These re-differentiated chondrocytes still have the capacity to form cartilage-like tissue in a high-density cell culture [35]. Furthermore, IGF-1 can block IL-1 β or NO-induced rapid dedifferentiation of chondrocytes and help maintaining chondrocyte phenotype [12, 40, 41]. In addition, IGF-1 blocks NO-induced apoptosis through PI3K pathway [42]. However, it has been shown that IGF-1 also increases the synthesis of COL-1 and COL-10 (an indicator of chondrocyte hypertrophy) [39, 42]. Therefore, much endeavor has to be directed to explore other factors that may have better re-differentiation potential.

Platelet-derived growth factor

Platelet-derived growth factor (PDGF) is a heat-stable positively charged hydrophilic protein. PDGF activates ERK and up-regulates TGF- β 1 and TGF- β 3 expression in human articular chondrocytes [20, 43]. Growth factor cocktail of TGF- β 1, FGF-2, and PDGF-BB has been

shown to increase human articular chondrocyte proliferation and maintain their chondrogenic potential [43]. More importantly, different from TGF- β 1 and FGF-2, PDGF-BB can promote the proliferation and extracellular matrix sulfation of resting chondrocytes *in vitro* without inducing a hypertrophic chondrocyte phenotype [44]. Combination of IGF-1 and PDGF-BB inhibits IL-1 β -mediated chondrocyte apoptosis, in part through suppression of PI3K/AKT pathway [45]. Therefore, addition of PDGF-BB to a monolayer culture system could be potentially used to maintain chondrocyte phenotype during *in vitro* chondrocyte expansion.

Transforming growth factor beta

The effects of transforming growth factor beta (TGF- β) on chondrocyte phenotype maintenance appear to be contradictory. For instance, one study demonstrated that TGF- β 1 is capable of stimulating COL-2 expression in monolayer culture of rabbit articular chondrocytes after 24 h treatment [46]. In contrast, after 6-day

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exposure, TGF- β 1 exerts an inhibitory effect on collagen synthesis without significant proteoglycan production change [47]. It is postulated that the chondrocyte status may influence the TGF- β 1 action on matrix synthesis. Moreover, the action of TGF- β 1 on matrix synthesis is species-dependent. Studies have demonstrated that TGF- β 1 can stimulate proteoglycan and COL-2 production during either expansion of goat articular chondrocytes or mouse precartilaginous stem cells in monolayer cultures [48, 49]. Although human articular chondrocyte dedifferentiation leads to decreasing TGF- β Receptor II (T β RII) expression level and subsequent TGF β response [50], the addition of TGF- β 1 during monolayer expansion of human articular chondrocyte results in the loss of COL-2 and ACAN while increases the level of COL-10 protein [51]. In addition, TGF- β 1 transduction pathway is also species-dependent. One study has shown that TGF- β 1 induces COL-2A1 expression in rat articular chondrocytes could implicate activation of ERK and subsequent activator protein 1 (AP-1) binding [52]. Another study reported that the COL-2A1 gene transcription is down-regulated by TGF- β 1 in rabbit articular chondrocytes through the increase of the Sp3/Sp1 ratio [53].

Recently, the effect of combination of TGF- β with other growth factors or gene intervention on chondrocyte phenotype gets more and more attention. For example, conjunct IGF-I with TGF- β 1 stimulates the dedifferentiated human articular chondrocytes to re-differentiate, with an increasing in the expression of the cartilage-specific proteins COL-2 and sex determining region Y box gene 9 (SOX-9) and down-regulation of COX-2 and MMP-13. Thus, they exert anabolic effects on chondrocytes and enhance the chondrogenic potential [12]. Also, adenoviral vector-mediated overexpression of TGF- β 3 and RNA interference of COL-1 drive dedifferentiated chondrocytes back to the differentiated phenotype in a three-dimension (3-D) culture system [54].

Bone morphogenetic proteins

Bone morphogenetic proteins (BMPs) are members of the TGF- β superfamily, which regulate a wide range of developmental processes and control the differentiation of several musculoskeletal tissues including bone and cartilage. BMP-2 and -7 have already been clinically used for bone regeneration [55-59]. Besides, several

BMPs, including BMP-2, -6, -9, -12 and -13, stimulate cartilage matrix macromolecules synthesis by articular chondrocytes [60-62], revealing their potential to promote cartilage repair. During monolayer culture, BMP-2 expression levels are diminished during passaging. The addition of BMP-2 to monolayer culture could slow down chondrocyte dedifferentiation and reinforce the maintenance of chondrocyte phenotype in long-term culture conditions [31, 63]. It was reported that BMP-2 has a stronger capability than TGF- β 1 to restore the character of chondrocytes. However, this re-differentiation is limited to a certain degree because COL-1 expression persists after the addition of BMP-2 or TGF- β 1 [64]. Combining BMP-2 with COL-1A1 siRNA effectively promotes the assembly of cartilage matrix in agarose hydrogel without production of COL-1 [65]. Simultaneous delivery of BMP-2 and SOX-9, two chondrogenic lineage-determining genes, results in significantly increased expression of chondrogenesis-related markers (COL-2 and ACAN) and glycosaminoglycan (GAG) matrix formation *both in vitro* and *in vivo*, compared with individual delivery of BMP-2 or SOX-9 gene [66]. Studies also have suggested that BMP-2 may have a better re-differentiation potential than IGF-I or TGF- β [64, 67]. Furthermore, co-transfection of IGF-I and BMP-2 can greatly increase the expression of COL2A1 and ACAN [39].

Although SMAD is the main signaling mechanism of TGF- β , the effect of BMP-2 on COL-2 expression is not mediated by SMAD pathway, as overexpression of SMAD-6 (an inhibitor of the canonical BMP-SMAD signaling pathway) could not block BMP-2-induced COL-2 pro-collagen expression in mouse chondrocytes [64]. Therefore, non-BMP-SMAD signaling pathway could play a key role in BMP-2-induced COL-2 expression in chondrocytes. This hypothesis is consistent with the results from a study that BMP-2 activates p38 MAPK, subsequently inhibits Wnt-7A/ β -catenin and eventually up-regulates SOX-9 and COL-2 expression [68]. Taken together, BMP-2 has a stronger potential to prevent dedifferentiation and induce re-differentiation in chondrocytes.

Fibroblast growth factors

Among the members of the fibroblast growth factor (FGF) family, FGF-2 and -18 have been implicated as key regulators in chondrocyte phenotype maintenance *in vitro*. The role of

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FGF-2 in chondrocyte dedifferentiation is in discrepancy. A series of studies have determined that FGF-2 functions as an anabolic inducer in animal articular chondrocytes. During monolayer expansion, the addition of FGF-2 reduces expression of fibroblastic molecules in calf articular chondrocytes. Furthermore, FGF-2 enhances chondrocyte response to BMP-2 in a 3-D culture system on poly (glycolic acid) scaffolds [69]. Of note, the role of FGF-2 in human articular chondrocytes appears to contradict its role in calf articular chondrocytes. Recent studies revealed that FGF-2 stimulates the human articular chondrocyte dedifferentiation in monolayer culture, suggesting it may serve primarily as a catabolic factor [70]. FGF-2-expanded chondrocytes are fully dedifferentiated but retain their potential of re-differentiation to hyaline cartilage in response to environmental changes [71]. Such discrepancies suggest that the function of FGF-2 in articular chondrocytes may differ fundamentally between species.

FGF-18 has been shown to promote early chondrocyte proliferation while inhibit the differentiation and matrix synthesis of chondrocytes [72]. Moreover, FGF-18 regulates chondrocyte proliferation and differentiation via binding FGF receptor 3 (FGFR-3) and activating ERK and p38 MAPK signal pathways [73]. A recent study verified that loss of chondrocyte phenotype is associated with increased FGF-18 expression level, indicating that FGF-18 may serve as a marker of dedifferentiation [74]. In this perspective, strategies to down-regulate the FGF-18 expression may contribute to the chondrocyte phenotype maintenance.

Signaling pathways in chondrocyte dedifferentiation

Extracellular signals evoked by ligand-receptor interaction enter into the intracellular compartment to initiate a series of responses and control multiple fundamental cellular processes such as proliferation, differentiation, survival and apoptosis. There are multiple cellular signaling pathways involved in chondrocyte dedifferentiation, such as MAPK, PI3K/AKT, PKC, WNT and Notch, as shown in **Figure 3**.

MAPK signaling

The MAPK pathway (p38MAPK, ERK and JNK) controls multiple fundamental cellular processes such as proliferation, differentiation, survival

and apoptosis. p38 MAPK plays a role in the maintenance of differentiated status of articular chondrocytes and induction of chondrocyte apoptosis. p38 MAPK positively regulates chondrogenesis while the ERK pathway functions as a negative regulator in staurosporine-induced re-differentiation [75]. It has been established that ERK signal transduction pathway is involved in the regulation of cell growth and differentiation. ERK-1 and -2 form the central component in the MAPK cascade. ERK-1/2 activation induces chondrocyte dedifferentiation and inhibits NO-induced apoptosis. In contrast, inhibition of ERK-1/2 subsequently blocks dedifferentiation [76]. However, a conflicting report showed that inhibition of ERK pathway can not block chondrocyte dedifferentiation [31]. ERK-1/2 activity in chondrogenesis is negatively regulated by PKC α , whereas ERK-1/2 and PKC α independently regulate dedifferentiation during chondrocyte passaging. Besides PKC, SH2 domain-containing transforming protein (SHC) negatively regulates ERK activation in the absence of growth factors in human cells. The adaptor protein SHC directly binds the ERK, thus preventing its activation in the absence of extracellular stimuli. The SHC-ERK complex restricts ERK nuclear translocation, restraining ERK-dependent transcription of genes [77]. A decrease in SHC-ERK interaction has been suggested as a marker for irreversibly dedifferentiated chondrocytes in tissue engineering [78].

Besides ERK-1/2 and p38 MAPK, the JNK signaling pathway is also reported to play a role in the differentiation of chondrocytes [15, 79, 80]. However, the results are controversial. JNK phosphorylates c-Jun, leading to the activation of AP-1 to suppress the expression of SOX-9, a major transcription factor regulating COL-2 expression. In addition, c-JNK/AP has been shown to function as downstream of WNT-3A-induced chondrocyte dedifferentiation [15]. A recent study demonstrated that JNK inhibitor suppresses miR-34-targeted Rac1 expression in chick chondroprogenitors, and Rac1 interacts with Rho to negatively modulate reorganization of the actin cytoskeleton, which is one of the essential processes for establishing chondrocyte-specific morphology [80].

PI3K/AKT signaling

IGF-1 binding to a receptor, namely IGF-IR, increases the activity of phosphatidylinositol

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3-kinase (PI3K), which up-regulates the levels of the phosphatidylinositol (4,5)-bisphosphate [PI(4,5)P₂] and phosphatidylinositol (3,4,5)-trisphosphate [PI(3,4,5)P₃]. The binding of AKT (also known as protein kinase B, PKB) to PI(4,5)P₂ and PI(3,4,5)P₃ allows tyrosine phosphorylation of the very important regulatory sites of AKT, resulting in the further phosphorylation of AKT substrates, such as mTOR [81]. PI3K/AKT signaling pathway plays a critical role in cell growth, proliferation and differentiation. Several studies have demonstrated that PI3K/AKT signaling pathway is not only responsible for the ability of IGF-I to increase chondrocyte proteoglycan synthesis [82], but also involved in blockage of the activation of p38 kinase and ERK-1/2 and inhibition of PKC α and PKC ζ induced by NO, which in turn suppresses dedifferentiation and apoptosis.

PKC

PKC is a family of protein kinases that control the function of other proteins through the phosphorylation of hydroxyl groups of serine and threonine amino acid residues on these proteins. In humans, the PKC family is divided into three subfamilies: conventional (α , β I, β II, and γ), novel (δ , ϵ , η , and θ), and atypical (ι and ζ). PKC α promotes chondrogenesis by activating COL-2 expression and blocks dedifferentiation of chondrocytes [83, 84]. PKC α expression is down-regulated during chondrocyte passaging, resulting in cellular dedifferentiation [84]. Therefore, PKC α functions as a critical signaling molecule promoting chondrocyte differentiation and maintaining its differentiated phenotype. A decrease in PKC α and ζ activities is required for both sodium nitroprusside (SNP)-induced dedifferentiation and apoptosis of rabbit knee joint articular chondrocytes. Although the dedifferentiation of chondrocytes during subculturing is not accompanied by any changes in PKC ζ expression and activity [76], inhibition of PKC ζ activity is required for SNP-induced dedifferentiation of chondrocytes [85].

WNT signaling

WNTs are a family of 19 morphogens that play a fundamental role in developmental processes ranging from embryonic morphogenesis to homeostasis of adult tissues. WNT pathway is divided into at least two branches: the canonical WNT/ β -catenin signaling and the β -catenin-independent “non-canonical” pathways.

In the canonical pathway, upon binding of WNTs (such as WNT-3A) to the co-receptor consisting of frizzled (FZD) and lipoprotein receptor-related protein (LRP), the dishevelled (DVL) is activated. Activated DVL inhibits the destruction complex, resulting in nuclear import of β -catenin and its subsequent binding with TCF (T cell factor)/LEF (lymphoid enhancer binding factor) family of transcription factors to initiate the transcription of target genes. The non-canonical WNT pathways include calmodulin-dependent protein kinase (CaMK) II pathway and c-Jun N-terminal kinase-mediated planar cell polarity pathway [86, 87].

Most of the cellular effects involved in WNT signaling are through the modulation of β -catenin. For example, WNT-3A and -7A stimulating β -catenin-mediated transcription involved in the regulation of chondrogenesis and dedifferentiation of primary articular chondrocytes [14, 15]. Accumulation of β -catenin and subsequent activation of the β -catenin-TCF/LEF complex induces articular chondrocyte dedifferentiation characterized by the suppression of COL-2 expression and the initiation of COL-1 expression. In addition, WNT-3A causes the expression of c-Jun and its phosphorylation by JNK, resulting in activation of AP-1, which suppresses the expression of SOX-9 [15]. Besides β -catenin, α -catenin also accumulates to interact with each other to form a complex in primary monolayer culture during chondrocyte dedifferentiation. This complex inhibits ubiquitin-independent degradation of α -catenin and β -catenin-TCF/LEF's transcriptional activity to block WNT-7A/ β -catenin induced dedifferentiation in chondrocytes [88]. Recent studies have demonstrated that α -catenin recruits the transcriptional repressor Gli3R to β -catenin to form a ternary complex, thus inhibits β -catenin's transcriptional activity and dedifferentiation of articular chondrocytes [89]. During WNT-3A-induced dedifferentiation of human articular chondrocytes, “non-canonical” CaMKII is also activated and reciprocally inhibits β -catenin-dependent pathways. Therefore, blockade of the canonical β -catenin pathway could induce dedifferentiation through de-repression of the CaMKII pathway [90].

Notch signaling

Notch signaling is a conserved pathway that plays a fundamental role in embryogenesis and

maintenance of adult biological processes [91, 92]. In mammals, the interaction of Notch ligands (Jagged 1 [JAG-1], JAG-2, Delta-like [DLL] protein, microfibril-associated glycoprotein [MAGP-1], MAGP-2, Notch like EGF-related receptor, and contactin) with its receptors (Notch1, 2, 3 and 4) leads to a series of cleavages mediated by ADAM proteases and γ -secretase complex, resulting in the release of intracellular domain of Notch receptor (ICN) from membrane [93, 94]. The active form of ICN fragment translocates into the nucleus and forms a complex with other transcriptional factors (such as recombination signal binding protein J [RBP-J]) and regulates downstream gene expression, such as hairy and enhancer of split 1 (HES1). Besides its important role in carcinogenesis, the Notch pathway has recently been found to be involved in the regulation of articular cartilage development [95], chondrocyte differentiation [96-98] and dedifferentiation during osteoarthritis [99]. Successive passaging of murine articular chondrocytes (MACs) results in chondrocyte dedifferentiation, which is accompanied by increased expression of Notch ligands (DLL-1, JAG-1) and HES1 rather than up-regulation of Notch1 receptor. Sustained activation of Notch1 signaling shows no effect on COL-2 mRNA expression, but decreases COL-2 protein levels in chondrocytes. MMP-13, the main MMP family member involved in COL-2 degradation, is up-regulated in the cells transfected with constitutive active forms of the Notch1 receptor [100]. These results demonstrate that Notch1 contributes to chondrocyte dedifferentiation via enhancing MMP-13-mediated COL-2 degradation. Surprisingly, the Notch inhibitor N-[N-(3,5-difluorophenylacetate)-L-alanyl]-L-phenylglycine t-butyl ester (DAPT) down-regulates COL-2 mRNA expression [101]. Therefore, besides Notch1, other Notch family members may also participate in the regulation of COL-2 expression.

Unlike the *in vitro* chondrocyte culture, chondrocytes reside separately in the cartilage lacuna and thus neighboring chondrocytes lack Notch receptor-ligand communication. MAGPs, the components of extracellular microfibrils, are able to dissociate from the Notch1 extracellular domain and activate the receptor [94]. In addition, MAGP-1 has been shown to interact with the chondroitin sulfate proteoglycan decorin in cultured fetal bovine chondrocytes [102], which suggesting that Notch signaling is in-

involved in chondrocyte dedifferentiation and OA. Therefore, we may infer that chondrocyte dedifferentiation and OA share many similarities.

Conclusions

During *in vitro* monolayer culture, chondrocyte dedifferentiation occurs very early even at the first passage [103]. Although it has been shown that the 3-D culture system can partially help chondrocyte re-differentiation from characteristics of dedifferentiated, it is still quite different from the native matrix *in vivo*. To date, it remains to be extremely difficult to prevent chondrocyte from dedifferentiation due to the lack of knowledge regarding this process. Given the complexity of growth factors and their complicated intracellular signaling pathways, the regimens of utilizing growth factors to maintain chondrocyte phenotype or using more specific inhibitors to block chondrocyte dedifferentiation need to be optimized. On the basis of crosstalk signaling pathways between anabolism and catabolism, future work should be directed toward identifying the intersection of signaling pathways that functionally impact on chondrocyte dedifferentiation. In addition, to design effective genetic modification-based treatment modalities to control the chondrocyte dedifferentiation for clinical applications, it is important to introduce no deleterious or off-target side effects. With more in-depth understanding of the molecular mechanisms underlying chondrocyte dedifferentiation, better treatment strategies may be on the horizon to combat chondrocyte dedifferentiation and maintain the chondrocyte phenotype and simultaneously enhance hyaline cartilage matrix production. At present, our lab work has begun to investigate the combination of growth factors as well as explore alternative pathways to avoid chondrocyte dedifferentiation for ACI. Although much remains to be explored, we believe that it would greatly contribute to maintain the chondrocyte phenotype if the balance of the anabolic and catabolic signaling pathways can be fine-tuned.

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Abbreviations

ACAN, aggrecan; ACI, autologous chondrocyte implantation; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; AP-1, activator protein 1; BMPs, bone morphogenetic proteins; CaMK, Ca²⁺/calmodulin-dependent protein kinase; c-JNK, c-Jun-N-terminal kinase; COL-1, type I collagen; COL-2, type II collagen; COX-2, cyclooxygenase 2; CTSK, cathepsin K; DVL, dishevelled; ECM, extracellular matrix; ERK, extracellular-signal-regulated protein kinase; FGF, fibroblast growth factor; FGFR-3, FGF receptor 3; FZD, frizzled; GAG, glycosaminoglycan; HES1, hairy and enhancer of split 1; ICN, intracellular domain of Notch receptor; IGF-I, insulin-like growth factor-I; IL-1 β , interleukin 1-beta; JAG-1, Jagged 1; LEF, lymphoid enhancer binding factor; MAPK, mitogen-activated protein kinase; MAGP-1, microfibril-associated glycoprotein; MMPs, matrix metalloproteinases; NO, nitric oxide; OA, osteoarthritis; PDGF, platelet-derived growth factor; PGE, E-series prostaglandins; PKC- α , protein kinase C alpha; PKC- ζ , protein kinase C zeta; PI3K, phosphoinositide-3 kinase; RA, rheumatoid arthritis; RBP-J, recombination signal binding protein J; shRNA, short hairpin RNA; SOX-9, sex determining region Y box gene 9; SHC, SH2 domain-containing transforming protein; SNP, sodium nitroprusside; TGF- β 1, transforming growth factor beta 1; TNF, tumor necrosis factor; 3-D, three-dimension; TCF, T cell factor.

Disclosure of conflict of interest

None to declare.

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