REVIEW

Differentiation of adult mesenchymal stem cells into chondrogenic cells using small molecules or microRNA

Onju Ham[1*], Chang Youn Lee[2*], Ran Kim[3*], Jihyun Lee[3], Min Young Lee[4], Jongmin Kim[5], Ki-Chul Hwang[6, 7], Woochul Chang[3]

[1]*Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, 250 Seongsanno, Seodamun-gu, Seoul 120-752, Republic of Korea*
[2]*Department of Integrated Omics for Biomedical Sciences, Graduate School, Yonsei University, 50 Yonsei-ro, Seodamun-gu, Seoul 120-759, Republic of Korea*
[3]*Department of Biology Education, College of Education, Pusan National University, Busan 609-735, Republic of Korea*
[4]*Department of Molecular Physiology, College of Pharmacy, Kyungpook National University, Daegu, 702-701, Republic of Korea*
[5]*Department of Life Systems, Sookmyung Women's University, Seoul 140-742, Republic of Korea*
[6]*Institute for Bio-Medical Convergence, College of Medicine, Catholic Kwandong University, Gangneung-si, Gangwon-do 210-701, Republic of Korea*
[7]*Catholic Kwandong University International St. Mary's Hospital, Incheon Metropolitan City 404-834, Republic of Korea*

* These authors contributed equally to this work.
Correspondence: Woochul Chang
E-mail: wchang1975@pusan.ac.kr
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Transplantation of mesenchymal stem cells (MSCs) into osteoarthritis (OA) and rheumatoid arthritis (RA) patients has been studied as a therapeutic tool for regeneration of damaged cartilage. MSCs have several beneficial effects, including immunomodulatory activity, and release various paracrine factors. Despite their abundant beneficial effects, transplantation of naïve MSCs is hampered by heterogeneous populations of differentiated and undifferentiated stem cells. However, transplantation of differentiated MSCs overcomes the problem of transplantation of naïve MSCs. Thus, to repair damaged tissue, a therapeutic strategy based on the use of differentiated MSCs is needed to treat RA or OA patients. Here, we summarize methods that can regulate differentiation of MSCs into chondrocytes by small molecules or miRNAs, and suggest the capacity of patient tissue-derived MSCs as a therapeutic strategy for treatment of OA or RA patients.

Keywords: microRNA; small molecule; differentiation; chondrocytes

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Introduction

Disruption of cartilage homeostasis is induced by combinations of biological mediators, which leads to the development of joint diseases such as osteoarthritis (OA) or rheumatoid arthritis (RA) [1, 2]. OA is characterized by disruption of the homeostatic balance between synthesis and degradation of cartilage components, including proteoglycans and other matrix components [3]. RA is induced by inflammation and catabolic driven cartilage loss [2]. An articular cartilage defect is an area of damaged or missing cartilage that is often caused by acute trauma because articular cartilage has a limited capacity for self-repair [4, 5]. Thus, regeneration of damaged cartilage is

required in chondrocytes.

Among the cell sources that have the ability to regenerate cartilage, mesenchymal stem cells (MSCs) have the capacity for treatment of articular cartilage defects [6]. MSCs have the capacity to repair damaged tissue and exert anti-inflammatory effects via cell to cell interaction or secretion of various factors. Thus, MSCs transplantation has been suggested as a therapeutic tool for treatment of joint diseases [7-9]. However, MSCs transplantation induces problems including acute graft-versus-host disease (GvHD) and autoimmune disease [10]. As a result, differentiated stem cells that have characteristics similar to host cells are needed for transplantation into damaged areas.

Differentiation of MSCs into specific cell types is regulated by intrinsic/extrinsic regulators and the extracellular niche [11]. Since small molecules have beneficial properties including rapid, reversible response and temporal regulators of protein function, their use has been suggested among several strategies to regulate cell fate [12, 13]. In addition, small molecules have been shown to regulate the fate of various tissue-derived MSCs via activation or inhibition of specific target proteins [14-16].

MicroRNAs (miRNAs) can also induce differentiation of MSCs into various cell types, including myoblasts, neurons, adipocytes, osteoblasts, and chondrocytes [17]. MiRNAs are a class of small non-coding RNAs that inhibit target gene expression via sequence specific interactions with the 3' untranslated regions (UTR) of mRNAs. MiRNAs also regulate target gene expression via degradation and/or repression of the translation of mRNA [18, 19]. Thus, it has been suggested that MSCs that have been differentiated into specific cell types by tissue-specific miRNAs be transplanted into patients for the treatment of joint disease. In the present review, we focused on the role of MSCs as a therapeutic strategy for treatment of OA or RA patients, as well as the methods required to induce differentiation into chondrogenic cells by miRNAs and small molecules. We also discuss the possibility for use of miRNAs or small molecules as a tool for differentiation of MSCs into chondrogenic cells.

Characteristics of adult stem cells used for treatment of diseases

Identification of Adult Mesenchymal Stem Cells

Adult stem cells are defined as cells that possess self-maintenance, proliferation and differentiation potential [20]. These cells are essential to physiological tissue homeostasis, renewal and regeneration of parenchymal cells after damage [21-23]. Adult stem cells generally reside as tissue-specific stem cells in many major organs in adult organisms, including bone marrow, heart, the nervous system, skin, skeletal muscle, cartilage, and fat [21, 22]. These characteristic suggest that all adult stem cells exist in unique microenvironments, or niches [23]. Niches contain heterogeneous cells and extracellular matrix proteins [23]. Adult stem cells also have plasticity, which means they can generate a fixed range of progeny when relocated to enable generation of specialized cells appropriate to their new niche [22]. In fact, adult stem cells are known to be surprisingly flexible in their differentiation repertoires in adult humans and rodents [22]. Adult stem cells are classified into hematopoietic stem cells, mesenchymal stem cells, and somatic stem cells. Mesenchymal stem cells (MSCs) are a subcategory of adult stem cells that can be roughly defined as mesenchyme for tissue [24]. MSCs are classified as umbilical cord-derived, bone marrow-derived, muscle-derived, cartilage-derived, tendon-derived, adipose tissue-derived, and synovial fluid-derived based on the mesenchymal tissue from which they originate [20]. MSCs are essential for physiological tissue homeostasis, renewal and regeneration based on their abilities for self-maintenance, proliferation and differentiation [21, 22]. Because MSCs have enormous values, they have been the object of extensive research for decades [25].

Therapeutic Potential of Adult Mesenchymal Stem Cells

MSCs are defined as highly adherent, proliferative undifferentiated multipotent cells with the potential to expand extensively *in vitro* [24, 26]. MSCs were first characterized in bone marrow, and it is now recognized that various adult components provide abundant MSCs, including bone marrow, peripheral blood, the heart, dental pulp, skeletal muscle, adipose tissue, and synovial fluid [27]. The cells have ability to differentiate into mesenchymal lineages such as bone, cartilage, tendons, adipose, muscle, marrow stroma, and neural cells [20, 28]. The multipotent potential of MSCs is an advantage, as is their easy isolation and culture, highly expansive ability in vitro, support of hemopoiesis, immunoregulation and secreted factors [24, 25]. MSCs also have the capacity to induce immunosuppressive effects during engraftment of MSCs in recipients, improving angiogenesis and preventing fibrosis [29]. This potential for feasible and safe administration has resulted in a broad interest in the clinical use of MSCs [27]. Taken together, these advantages make MSCs attractive and promising for application in many different fields, including tissue engineering and clinically-viable cell therapy, and there are now numerous trials being conducted to investigate their use in a wide range of diseases including stroke, heart failure, liver failure, and osteogenesis imperfecta [22, 24, 27, 30]. Chen *et al*. confirmed that symptoms of ischemic stroke were

moderated by transplantation of MSCs. Specifically they showed that MSCs could survive, migrate, and differentiate into parenchymal cells in adult mice brains after ischemia. Transplantation of bone marrow MSCs has also been shown to reduce ischemic stroke-induced behavioral deficits in mice [31]. Qin *et al* found that osteogenesis was effectively induced by attaching MSCs within biomaterial scaffold, including hydroxyapatite and tricalcium phosphate [32]. Morishita *et al* demonstrated the effects of MSCs-bioscaffold applied to treat bone defects after tumor resection at the distal tivia by attaching autologous MSCs to hydroxyapatite to induce bone reconstruction [33].

Table 1. Summary of small molecules regulating differentiation of stem cells

Small molecule	Cell type	Function	Target	Reference
H-89	Human bone marrow-derived MSCs	Stimulation of differentiation	PKA inhibitor	39
Kartogenin	Human bone marrow-derived MSCs	Stimulation of differentiation	disrupts core-binding factor β subunit	40
Harmine	ATDC5	Stimulation of differentiation	Inducer of CCN2	42
SB216763	ATDC5	Inhibition of differentiation	GSK-3β inhibitor	43
Pam3Cys	Murine MSCs	Inhibition of differentiation	TLR inhibitor	44

Table 2. Summary of miRNA induced differentiation into chondrocytes

MicroRNA	Cell Type	Function	Target gene	Reference
miR-23b	Human SF-MSCs	Stimulation of differentiation	PRKACB	29
miR-101	Chicken limb mesenchymal cells	Stimulation of differentiation	Dnmt3b	54
miR-194	Human adipose-derived MSCs	Stimulation of differentiation	Sox5	55
miR-455	ATDC5	Stimulation of differentiation	Smad2	56

Differentiation of MSCs into chondrocytes by miRNA or small molecules

Differentiation of MSCs into Chondrocytes by Small Molecules

Several reports have shown that small molecules have the capacity to control proliferation, differentiation, apoptosis, and migration of cells. Differentiation of MSCs through the use of small molecules was first reported in 1978 by Strickland and Mahdavi, who revealed that cell reprogramming is induced by retinoic acid or cyclic AMP compounds [34]. In addition, chemical compounds can target specific enzymes or proteins by which maintenance, differentiation, and reprogramming of MSCs are controlled [35-37]. Moreover, regulation of stem cell fate by small molecules has enabled development of new drugs using the patient's own cells residing in different tissues or organs to treat diseases [38]. Several studies have shown the differentiation of MSCs into chondrogenic cells by treatment with small molecules. Ham *et al.* reported that treatment with H-89, a protein kinase A (PKA) inhibitor, promoted chondrogenic differentiation of bone-marrow derived MSCs [39]. Johnson *et al.* reported that small molecule kartogenin promotes chondrocyte differentiation. Specifically, they found that, in addition to its chondro-protective effects in vitro, it is efficacious in OA animal models [40]. Moreover, harmine induced differentiation into chondrocytes in ATDC5, a line of mESCs [41, 42]. However, some studies have suggested that inhibition of specific pathways such as GSK-3β or TLR prevents differentiation of stem cells into chondrocytes by treatment of SB216763 or Pam3Cys [43, 44]. Taken together, these reports suggest that small molecules have the potential for differentiation of stem cells into chondrocytes via regulation of specific proteins or signal pathways. Thus, differentiation of stem cells into chondrocytes is a good strategy for development of novel treatments for OA or RA patients.

MicroRNAs for Differentiation of MSCs into Chondrocytes

MicroRNAs play essential roles in various biological processes including development, proliferation, death and differentiation. Many studies have recently reported that a variety of cells including cardiac muscle cells [45], nerve cells [46], muscle cells [47], liver cells [48] undergo differentiation in response to microRNA treatment [49].

miRNAs are small non-coding single strand RNAs that play important roles as key regulators of gene expression via post-transcriptional regulation of target mRNA or translational inhibition of target proteins by associating with the 3'-untranslated region (3'-UTR) of target genes [50]. The expression of miRNAs changes during differentiation of MSCs into chondrocytes. For example miR-574-3p, and miR-335 are highly upregulated in MSCs [51, 52]. Additionally, MiR-24 and miR-199a increased by over five-fold during differentiation into chondrocytes and osteoblasts [53]. Several studies have suggested methods for inducing differentiation into chondrocytes by overexpression of miRNAs. For example, Ham *et al.* found that miR-23b facilitated differentiation of human SFMSCs into chondrocytes, and that it increased expression of the chondrocyte markers, collagen type II, collagen type X, and Sox9. Conversely, miR-23b reduced expression of hypertrophic markers of MMP-2 and MMP-9 [29]. Kim *et al.* also reported that two non-coding RNAs, miR-101 and HOTTIP, induced chondrogenesis of limb mesenchymal cells by targeting Dnmt3b. Their study revealed that HOTTIP, non-coding RNA, and miR-101 were important to treatment of arthritis [54]. In addition, miR-194 and 455 reportedly induced chondrogenesis in ASC and ATDC5 cells [55, 56]. Based on these studies, miRNAs are one of the crucial factors involved in induction of differentiation of MSCs into chondrocytes. As a result, microRNA will be the focus of new therapeutic strategies for treating arthritis in future studies.

Therapeutic strategy for OA or RA patients

Transplantation of Synovial Fluid-derived Mesenchymal Stem Cells from Patients

OA and RA are the most common forms of articular disorders. OA occurs throughout the joint and ultimately causes articular cartilage loss and progressive joint degeneration [57]. RA is an autoimmune disease characterized by systemic inflammation and persistent synovitis that destroys the joint via progressive cartilage degeneration and bone alterations [58]. Injury of articular cartilage has been treated by perichondrium transplantation and autologous chondrocyte implantation, but these approaches are limited by their invasiveness, graft site morbidity, low number of harvested chondrocytes, and loss of functionality after several passages of *in vitro* culture [59]. Autologous chondrocyte transplantation, which has been used widely since 1994, is the first method of treatment developed by Matts Brittberg [60]. This method facilitates the regeneration of damaged cartilage by autologous chondrocyte transplantation of cells cultured in vitro. This technique requires a culture time of about 4 weeks to obtain a sufficient number of cells; however, the use of stem cells has been proposed to solve these limitations. SF micro-environment of the diseases can be to induce response of cytokines and chemokines, and inflammatory, which are able to enhance proliferative response of SF-MSCs [59]. Therefore, use of SF-MSCs from patients might be an alternative therapeutic application for OA and RA owing to the abundance and accessibility of human synovial fluid, as well as their efficacy and safety [29]. SF-MSCs represent an attractive therapeutic candidate for cartilage repair in response to conditions such as osteoarthritis (OA) and rheumatoid arthritis (RA) therapy because of their multipotency, *ex vivo* proliferation capacity, and high chondrogenic potential relative to MSCs from other tissues, as well as their ability to expand over a short time period after joint disease and injury [59, 61, 62]. Previous studies have demonstrated that the use of SF-MSCs for OA and RA could mitigate these disorders. For example, intra-articular injection of MSCs from synovium enhanced cartilage regeneration in a rabbit cartilage defect model [64] and contributed to meniscus regeneration in a rat meniscus defect model [65]. Moreover, engrafting of SF-MSCs was shown to have the potential to increase the number of intra-articular MSCs, resulting in their migration to the site to participate in repair response [64].

In conclusion, this review suggests that MSCs can be differentiated into chondrocytes through the use of miRNAs or small molecules. Moreover, transplantation of SF-MSCs from a patient's own cells that have been induced to differentiate into chondrocytes has the potential to treat damaged tissue.

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