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Review Article

Involved Molecular Mechanisms in Stem Cells Differentiation into Chondrocyte: A Review

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Abstract
Stem cells are unique biological cells that can differentiate into specialized adipocytes. In mammals, there are two broad types of stem cells: embryonic stem cells that break away from the blastocyst cell proliferation, and adult stem cells that are found in different tissues. Mesenchymal Stem Cells (MSCs) are multipotent cells that are one of the most important adult stem cells. Due to their high proliferative capacity and the proper self-renewal ability, they have provided a powerful and promising source to use in the field of repair plaque. Also, MSCs can differentiate into several cell types, such as: osteoblasts (adipocytes), chondrocytes (chondrocytes), adipocytes (adipocytes) and myocytes (muscle cells). Because of the importance of MSCs as a source of autologous transplantation in the field of regenerative medicine, in-depth studies of involved cell and molecular signaling cycles are needed. These cycles are the reason in which these cells are able to differentiate into other cell types. Also, the molecular changes that occur during these cells differentiation are needed to be closely examined. The role of cytokines, chemokines, and transcription factors on the process of differentiation of these cells is considered significant. The differentiation of MSCs into other cell lines is manipulated and stimulated by specific transcription factors associated with specific cell lines, thus, the important role of non-coding small mRNAs (miRNAs) is increased as a result. In the following study, the process of differentiation of MSCs into chondrogenic lineage and the effect of several miRNAs on the regulation of the process of differentiation into adipose-derived stem cell cartilage have been scrutinized.
Keywords: Cartilage Stem Cell, Differentiation, Molecular Mechanism, Transcription Factors
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Introduction

The aim of this review is to demonstrate the roles on molecular mechanisms in the differentiation of stem cells and Chondrocyte and to review the most important molecules in these pathways which enable us to evolve new therapeutic and other interventions regarding these mechanisms.

The term stem cell means what that has the potential to perform multiple cell divisions and has unique abilities to produce exactly the same cell in a process called self-renewal as well as differentiation to different cell types, which are the vital factors in the early fabrication of tissues and repair of damaged tissues (tissue repair).[1] Stem cell types are differentiated by tissue, origin, rate of proliferation, cell differentiation, evolutionary stage, and the type of genes expressed. All stem cells respond to self-regulating, differentiating, and controlling cell cycle regulators, regardless of source tissue with similar mechanisms. The cell protection processes, DNA damage repair, apoptosis signaling pathways, and cellular senescence are also tightly controlled in all stem cells by similar mechanisms.[2]

There are two types of stem cells in a broad range: embryonic stem cells and mature stem cells. Embryonic stem cells are obtained from the inner cell mass of the *blastocyst*, a mainly hollow ball of cells that, in the human, forms three to five days after an egg cell is fertilized by a sperm. Embryonic stem cells are *pluripotent*, meaning they can give rise to every cell type in the fully formed body, but not the placenta and umbilical cord. These cells are incredibly valuable because they provide a renewable resource for studying normal development and disease, and for testing drugs and other therapies. Mature stem cells (adult stem cells or tissue specific stem cells) are more specialized than embryonic stem cells. Typically, these stem cells can generate different cell types for the specific tissue or organ in which they live in. The present study aims at analyzing the differentiation of embryonic stem cells to Chondrocyte from all possible molecular aspects that might

have been neglected in previous studies.

Adult Stem Cell Differentiation Toward Cartilage Lineage

Bone marrow MSCs are the most source of MSCs. Besides bone marrow, multiple tissues have been reported to contain MSCs. These include adipose tissue, trabecular bone, periosteum, synovial membrane, and skeletal muscle, as well as teeth and umbilical cord. Furthermore, some researchers have paid special attention to synovial membrane as a potent source of stem cells with good chondrogenic potential.

Bone marrow contains two types of hematopoietic and MSCs.[3] MScs are multipotent with high proliferation and self-renewal potential as well as the potential to differentiate into different cell lines including osteoblast, chondrocyte, adipocyte, endothelial cells, and neuron cells. The most important feature of these cells is the ability to form colonies resembling bone and cartilage.[4]

Also, mesenchymal cells are high-powered cells that are highly capable of producing connective tissue such as cartilage, bone, tendon, ligament, and stroma brain during organogenesis. These cells are one of the most important stem cell sources in remedial medicine.[5] Generally, these stem cells vary based on tissue, origin, rate of cell proliferation and differentiation, evolutionary stage, and type of genes expressed. All stem cells respond to self-regulating, differentiating, and controlling cell cycle regulators, regardless of source tissue, with similar mechanisms. The cell protection processes, DNA damage repair, apoptosis signaling pathways, and cellular senescence are also tightly controlled in all stem cells by similar mechanisms.[2]

Tissue cartilage is compact, flexible, oily and non-self-healing in nature. Cartilage is one of the specialized tissues of the body as it is deprived of any veins, nerves and lymph.[6] The precursor cells used for cartilage that have cartilage potentialities include MSCs derived from bone marrow and adipose tissue, Adenovirus, nodule, and muscle.[7] Conventional cell-based clinical methods to treat chondrogenic injury and rebuild cartilage are solely based on chondrocyte cells. However, much effort has been made to develop articular cartilage naturally in vitro environments through 3D culture, bioreactors, and mechanical, biochemical stimulation. During these experiments, in order to decrease the disadvantages of chondrocyte culture, the tendency has been to use stem cells as approved by the US Food and Drug Administration.[8]

When damaged, the tissue may heal itself using stem cells that can repair themselves. This is the case if the lesions are limited and there is tissue healing ability.[9] Therefore, proliferation or self-renewal is the ability of cells to produce identical copies of themselves by dividing the mitosis over a given period of time (the entire life span of a living being) (Figure 1).

In the differentiation of MSCs, two distinct sections are considered. In the first (the stem cell compartment), the cells are stable and grow in the G0/G1 stage until proper stimulation (e. g, growth factor) is established. By stimulation, the cells divide into a non-symmetric cornea, and the resulting daughter cells, one resembling the parental cell, and the other precursor cell become more restricted with differentiation. The precursor cells are diverged asymmetrically, and the trivalent and divalent cells result in a morphology similar to that of the multivalent cells. These cells are still attached to the stem cell compartment. The compartment of committed cells consists of the cells initially capable of differentiating into a single cell line, and the extraction of mesenchymal cells was only from the bone marrow, but as time went on it became clear that this type of stem cell could be derived from tissues extracted from this cell such as adipose, bone, dental pulp, and cord blood.[10,11]

Differentiation of Bone Marrow-Derived MSCs into Cartilage

Studies have been carried out on the capability of cartilage differentiation to bone marrow in which the presence of cells with scaffolds of different chemical composition, different environmental and physical conditions such as mechanical loads or different chemical conditions such as the presence of biomolecules have been reviewed. One of the most important sites of differentiation of these types of Chondrocyte is serum-free medium but with dexamethasone, ascorbic acid and sometimes growth factor. The most important characteristic of chondrogenic differentiation are gene expression, Sox9 type II collagen, and organic which can be dramatically increased by stem cell differentiation to Chondrocytes.[11]

Differentiation of Stem Cells Derived from Adipose Tissue into Cartilage

The process of extracting stem cells from adipose is less invasive and also more cells can be extracted. Adipose stem cells are different with bone marrow cells in a way that bone marrow stem cells have smaller cellular body and have different gene expression and cell surface receptors, and in addition, can handle more passages in the laboratory. Also, adipose stem cells reduce Transforming growth factor beta(TGF-β) compared to bone marrow, and need factors such as bone morphogenetic protein (BMP) to differentiate cartilage.[11]

Differentiation of Synovial Tissue-derived MSCs into Cartilage

The non-adrenal surface of the joints is covered with a thin membrane called the synovial membrane. The most important task of this tissue is to preserve synovial fluid, which is the source of articular cartilage. The expression of chondrogenic markers such as Sox9 agricane and Chondrogenic Oligomeric Matrix Protein (COMP) in these cells are greater than 1% to

3% in adult articular Chondrocyte and also have higher potential for synovial MSCs. In synovial derived stem cells, the presence of growth factors such as TGF-β, insulin-like growth factor (IGF-1) and the fibroblast growth factor dramatically increase cartilage differentiation.[12]

Differentiation of MSCs Derived from Other Sources into Cartilage

Mesenchymal stem cells are derived from many mesenchymal or non-mesenchymal tissues, including the periosteum, trabecular bone, cord blood, amniotic fluid, and muscle skeleton.[12] The periosteum-derived MSCs have demonstrated the ability to chondrocyte and chondrocyte proliferation in different animals and the body of animals. In contrast to MSCs, cord blood is a good option as an allogeneic source that is easily accessible when these cells are in the body with different hydrogels, displaying high cartilage differentiation. These type of stem cells have the ability to differentiate into different classes, and by applying differentiating conditions to the cartilage they increase the expression of aggrecan.[12]

The Effect of Chemical Factors on the Differentiation of MSCs into Cartilage

The chemical constituents are BMP, TGF-β, and IGFs: BMPs growth factor and secrete differentiation is present in mineralized bone. In mammals, three types of BMP are known, but only type I and type II receptors bind to BMPs and cause phosphorylation. The BMPs act as regulators of bone and cartilage growth and are also involved in the repair of the adult skeleton. It has been shown that 2-BMP can enhance cartilage differentiation, but its mechanism is still unclear.[13] One of the problems associated with MSCs is the reduction of their differentiation potential over time.[14]

The Effect of Scaffold Chemical Composition on the Differentiation of MSCs to Cartilage

One way to improve bone marrow cell differentiation to cartilage is to induce cartilage by the matrix of autologous, which is stimulated by cartilage stimulated with an active scaffold such as type 1, 2 or chitosan at the site of cartilage injury after bone marrow treatment. This type of scaffold improves the differentiation of bone marrow-derived MSCs into cartilage and restores cartilage. In the present research, synovial stem cells were cultured on this matrix and the result of cartilage differentiation was observed.[15]

Physical Factors Influencing Differentiation of MSCs to Cartilage

One of the most important physical messages that alter the fate of the cell is the cell shape that can be controlled by mechanical forces or matrix geometry.[16] It is clear that the cells lose their natural phenotype after extraction. The cell membrane has chemical and topographic parameters that identify the firmness and structure of the substrate. In addition to affecting the shape of the matrix, other physical factors also influence cell fate, such as surface stiffness which alter cellular junctions and stresses on the connecting parts such as the cytoskeleton, and activates a specific trajectory in the cell through differentiating it which results in changing the cell behavior.[16]

Molecular Mechanism of MSCs Differentiation to Cartilage

Growth Factors

They play an important role in regulating the expression of collagen type 2 and 9, 10, 11, aggrecan, and cartilage-binding proteins that serve as markers for the identification of Chondrocytes.[17]

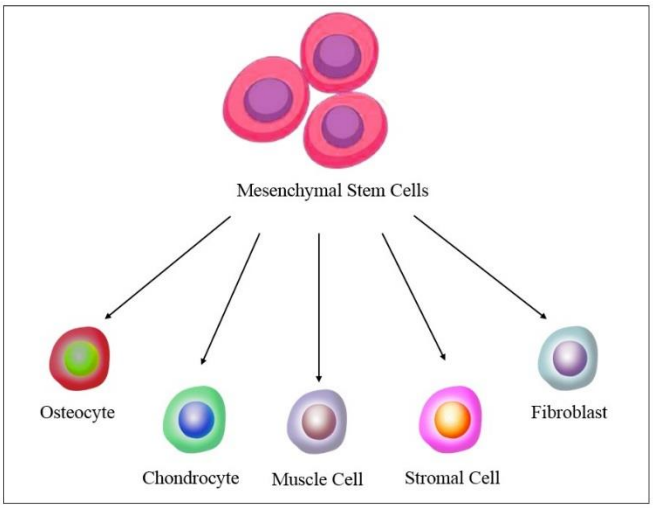

Figure 1. Theoretical Model of Mesenchymal Stem Cell Differentiation Potential. Mesenchymal stem cells can be differentiate into osteocytes, chondrocytes, muscle cells, stromal cells and fibroblasts.

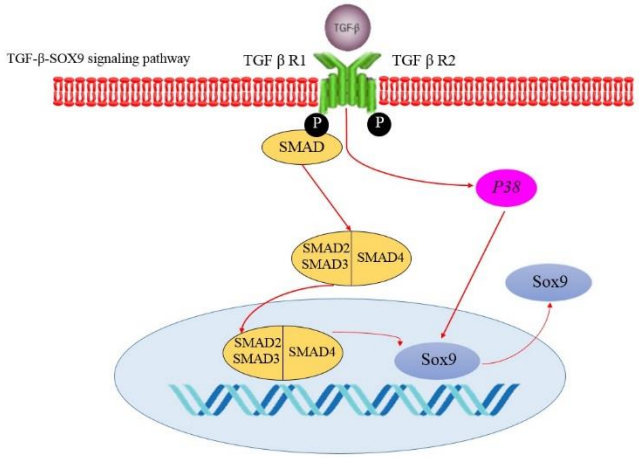

Figure 2. Function of TGF-β. TGF-β ligands binds to its receptors and therefore phosphorylates smad protein in which leads to smad complex formation that can triger its target gene expression.

Beta Transient Growth Factor (TGF-β)

Among the growth factors, the most well-known stimulus of cartilage production is the TGFβ beta superfamily. This superfamily has more than five members, of which TGF-β1, TGF-β2, and TGF-β3 are generally regarded as potent stimuli in type II collagen synthesis and proteoglycans and cartilage differentiation[18] (Figure 2).

The TGF-β1 reduces the process of regulating adipose-derived stem cell signaling cycles by activating receptors located across the TGF-β type I and II membranes.[19] As a result of ligand binding, TGF-β activates type I receptors of type II, which phosphorylates downstream Smad and thereby enhances the transcription of genes involved in the production of cartilage including Sox9. The small molecules type 2, 3, and 4 are transferred to the nucleus and activate the transcription of certain genes 16, which contains the p38MAPK cycle. The inhibition of p38 activity inhibits the expression of genes specific for cartilage production and matrix production.[20]

The TGF-β3 combinations can initiate the process of differentiation into adipose-derived stem cells. Unfortunately, TGF-β3 can also induce hypertrophy and calcification of Chondrocytes, and subsequently, a regulatory signal during endothelial bone formation of the small protein G that develops a Racl.[21]

Racl is a small G protein that acts as a positive regulator involved in hypertrophy, maturation, and calcification of chondrocytes.[22] Racl repression modulates cellular hypertrophy activity without affecting the ability of adipose-derived stem cell cartilage.[23]

Hoxa

The decrease in Hoxa 2 gene expression was determined during the process of MSC differentiation to cartilage, and further forced expression of Hoxa 2 resulted in inhibition of MSC differentiation process. Another study also concluded that HOXD9, HOXD10, HOXD11, and HOXD13 factors inhibit the differentiation of MSC into cartilage.[24]

Zinc-Finger Protein 145

Zinc-finger protein 145 (Zinc-finger protein 145) is a transcription factor that plays a key role in the process of differentiation of MSCs into chondrocytes.[25] The role of ZNF 145 in MSCs reduces the process of differentiation of MSCs into Chondrocyte lines. Whereas, overexpression of ZNF145 increased SOX9 expression and cartilage formation.[26]

Smad

One regulator of the process of chondrogenesis is MSCs. A study showed that Smad 3 by binding to the Sox9 transcription factor disrupted the differentiation process of these cells into the chondrogenic line.[26]

Yap

As a regulator of the process of differentiation of MSCs into bone, it also has an inhibitory effect on the process of MSCs differentiation to cartilage.[27] Also, STAT3 plays a key role in the process of differentiation of cartilage-derived MSCs through the activation of the STAT3 cycle by cytokine 6-IL.[28]

Want 11

Expression of more than 11 Wnt expresses cartilage-related gene regulators.[29] In addition to Want 11 along with TGF-β, it stimulates the progression of the process of mesenchymal stem cell cartilage.[30]

Sox9

An important transcription factor that plays a major role in differentiating chondrogenic MSCs is sox9 (SRY-related high mobility group-box gene 9). Sox9, by binding to the type 9 collagen gene promoter and forming trans-activating complexes with other proteins controls the expression of type 9 collagen[31] This transcription factor is a key regulator of chondrocyte differentiation and cartilage formation that can delay the maturation of hypertrophy at certain stages of chondrogenic differentiation.[31] (Figure 3).

This factor is required for the expression of type II collagen genes and other cartilage-specific matrix proteins. Two other members of the Sox family, SOX6 and L-SOX5, are not only expressed early in the mesenchymal compaction stage, but also co-expressed with Sox9 during chondrogenic differentiation.[32]

Bone Morphogenetic Proteins (BMPs)

It is a large subset of five polypeptides that plays a key role in the process of cartilage and bone formation during skeletal development. Several BMP proteins, including type two, four, six, seven, thirteen and fourteen stimulate the process of cartilage differentiation protein and enhance the synthesis of collagen type II and agonists by chondrocytes in vitro.[33]

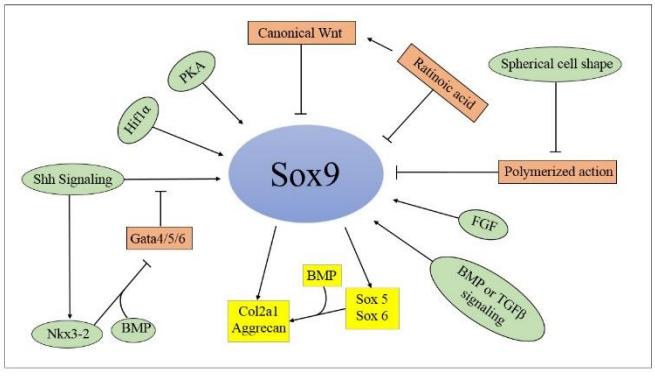

Figure 3. Sox9 transcription factor function. Some signaling pathways including PKA, Hif1α, shh signaling, FGF, BMP can trigger Sox9 expression and therefore expression initiation of Sox5, Sox6 and Col2a1. In addition, some other pathways such as canonical Wnt and some molecules such as retinoic acid and polymerized actin can inhibit Sox9.

FGF

The FGF family consists of 22 structurally related proteins that bind several FGFRs. The FGFs, and their binding to FGFRs activates multiple signal-transduction cycles. Among them, the best characterized cycle is the Ras-mitogen activated protein kinase cycle.[19] The FGF-2 induces the cartilage-building potential by expressing cadherin-N, FGFR2, and 9 Sox transcription factor in adipose-derived stem cells and also preserves the process of cell proliferation.[34]

Matrix-Cell Binding

An important regulator is survival, self-renewal, and stem cell differentiation.[35] In general, the cartilage manufacturing process requires a three-dimensional culture system. The type of biomaterials used as scaffolds appears to be the major Adipose derived stem cell cartilage during the process, and the size of the scaffold pore and its hardness should be considered as determinants.[36] Natural and favorable polymers have cell degradation properties in terms of cell attachment, proliferation, and biodegradability enabling cell replacement when scaffolding is degraded.[36] In addition, cell culture on natural scaffolds drives cells to produce cartilage-like compounds including type II collagen, Agericane and Glycosaminoglycan.[37] Adipose derived stem cells differentiate into cartilage-like cells when cultured in 3D matrix or in the presence of growth factors such as TGF-β.[38]

Non-Coding Small mRNA Molecules (miRNAs)

In the RNA silencing process, as well as the post-transcriptional regulation of gene expression, the role of miRNAs in the process of differentiation of adipose-derived stem cells into cartilage has also been addressed (Figure 4). Several miRNAs are involved in regulating the molecular cycles of the cartilage differentiation process by stimulating or inhibiting the cartilage manufacturing process. Some miRNAs are highly expressed during cartilage development.[39] Overexpression of miR-140 plays an essential role during cartilage development by regulating some of the target genes including the four HDAC and Smad3 genes (important in the process of cell differentiation) in the mouse animal model.[40] Also, miR-574-3p is overexpressed in the early stages of cartilage and maintains its increase throughout the differentiation process. Initial stimulation of miR-574-3p directly affects the down-regulation of Sox9 and RXRα.[41]

In contrast, miR-199 expression decreased during the early stages of cartilage production and paralleled by an increase in Smad1 expression, but the miR-199 expression level was successfully increased. The suggestion that miR-199 is essential for the late stages of cartilage production has been deduced from the process of hypertrophy and chondrocyte maturation.[42]

It has also been suggested that overexpression of 145 miR- and 495 miR- inhibits Sox9 expression levels after the transcription process reduces expression in some specific cartilage markers such as COL2A1 and COL9A2.[43] The MiR-335-5P is another miRNA involved in the induction of chondrogenesis that is overexpressed during the process of differentiation of murine bone marrow-derived MSCs and stimulates the process of overexpression of Sox9 and other markers involved in the process of differentiation.[44]

Studies have also shown that miR-30a is overexpressed during the process of cartilage production by the target DLL4, which is a Notch ligand that modulates cartilage differentiation.[45] In the process of differentiation of adipose-derived stem cells, at least two miRNAs have been studied,

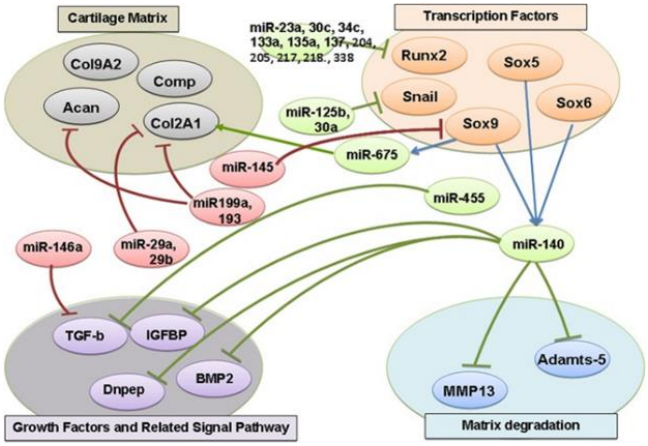

Figure 4. miRNAs involved in cartilage differentiation. miRNAs are responsible for inhibition of cartilage matrix, transcription factors, growth factor and related signals pathway and matrix degradation molecules mRNAs.

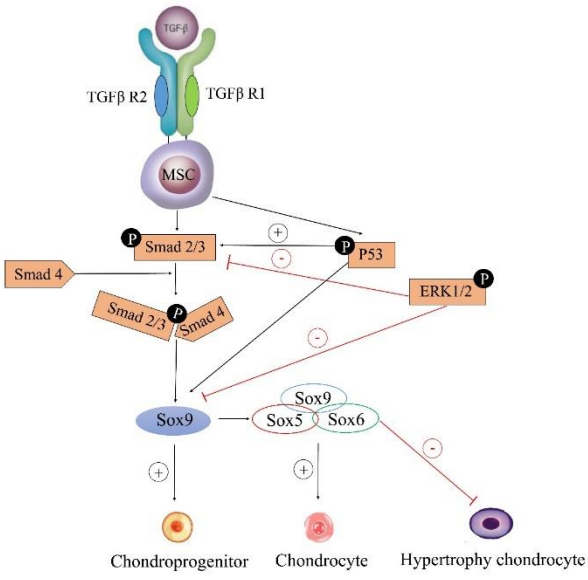

Figure 5. Factors involved in cartilage differentiation. Following binding of TGF-β to its receptor, some molecules such as Smad proteins complex, p38 and ERK1/2 can affect sox5, sox6 and sox9 molecules thus influence on cartilage differentiation.

In the process of differentiation of adipose-derived stem cells, at least two miRNAs have been studied, of which eight were expressed in differentiated cells in differentiated cells compared with undifferentiated cells and the other eight have shown decreased expression.[46]

The miR-193b inhibits Smad3 phosphorylation and inactivates the TGF-β signaling process and negatively regulates the early stages of cartilage production.[47]

In addition, down regulation of miR-490-5P has the potential to inhibit cartilage differentiation by suppressing BMPR2 expression. Thus, overexpression of miR-490-5P is associated with increased expression of chondrogenic markers such as COL10A1 and COL2A1 and, if applicable, overexpression of miR-490-5P directly targets BMPR4.[46] Also, some studies have shown the role of miR-194 during the process of adipose-derived stem cell cartilage. The miR-194 directly expresses Sox5, one of the key factors involved in cartilage-related transcription.[48]

Sox5 activates COL2A1 genes, if there are any, and plays an important role in regulating extracellular matrix accumulation.[49] During induction of the process of stem cell differentiation to cartilage, a decrease in miR-194 levels stimulates an increase in Sox5 expression, thereby facilitating the process of adipose-derived stem cell cartilage.[48] Another study reported that the increased expression of miR-92a during the process of differentiation of adipose-derived Adipose-derived stem cells induced an increase in the expression of COL9A2 and IFN. The positive involvement of miR-92a in the production of cartilage seems to be mediated by an increase in the expression of PI3k-Akt and mTOR.[50]

Processes of Stem Cell Differentiation to Chondrocyte

The process of differentiating stem cells into cartilage is carried out in four consecutive stages, with several chondrogenic genes expressed at each step including the most important genes expressed in type I and type I collagen, SOX4 and BMP2[51] (Figure 5). Also, genes HAPLN1, COMP, collagen XI, and SOX 9 are expressed in the second stage of this process. The most important genes expressed in stage III are Chondroitin and -N-cadherin and in the latter stage genes such as aggrecan, Alkaline Phosphatase, collagen type X and IX, fibromodulin II and fibronectin are expressed. At first, MSCs are involved in chondrogenesis by paracrine factors that increase the expression of key transcription factors, which activates chondrogenic genes. Secondly, the committed MSCs are compressed into dense nodules and differentiated into chondrocytes. During the third stage, Chondrocytes rapidly proliferates and increases their cytoplasmic content and secrete a large amount of extracellular matrix specific for cartilage. After this stage, the expression of cell profiles changes and collagen type X and fibronectin are secreted. The differentiation of chondrogenic precursors is characterized by the degradation of chondrogenic matrix containing collagen XI, X, IX, Agranic, and Sox 9 expressed at the compression stage. Chondroblasts then mature and participate in the morphogenetic processes that begin to form a cohesive mold at the sides of each joint.[32]

Conclusion

In order to fulfill the overall aim of this study and to get familiar with the specificity of the cartilage production process in MSCs, one should be notified that some of them are obtained by the use of complex inducer protocols with highly potent stimulating and extrinsic factors. Also, this strong stimulus from the suitable signaling cycles occurring in vitro as well as the process of differentiating of cartilage-derived MSCs need to be evaluated in animal models.

Authors' Contributions

All authors contributed equally to this study.

Conflict of Interest Disclosures

The authors declare that they have no conflict interests.

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