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Advances in the Influence of Wnt on Mesenchymal Stem Cell Differentiation

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Abstract

Mesenchymal stem cells (MSCs) possess self-renewal ability and high differentiation potential. It can be used to construct in vitro models to assist with pathological and pharmacological research, as well as applications in tissue engineering. The core process in the osteogenic differentiation of MSCs is osteogenesis, which is the process of bone tissue formation primarily carried out by osteoblasts. Chondrocytes and skeletal muscle cells are also involved in the process of bone injury repair. As a group of highly conserved signaling proteins, Wnt family proteins affect the direction of MSC differentiation through autocrine or paracrine mechanisms. This review summarizes the influence of Wnt in differentiation process, as well as the relevant factors in the Wnt signaling pathway for directed differentiation during cell culture, aims to provide new solutions and guidance for precise treatment.

Keywords

Wnt, mesenchymal stem cells, directed differentiation

1. Introduction

Growth factors are a class of polypeptide or protein biological signaling molecules that can regulate cell growth, division, differentiation and metabolism, utilized to induce the differentiation of mesenchymal stem cells (MSCs). Osteogenesis-related growth factors include VEGF, BMP and Wnt family proteins.

The osteogenic differentiation of MSCs holds great potential for applications in the medical field. Statistical data show that from 1990 to 2021, the incidence and prevalence of spinal fractures in China have continued to rise, making fracture treatment and related rehabilitation increasingly important[1]. Currently, common treatment such as internal fixation screws often pose difficulties in removal after surgery and may lead to complications such as bone necrosis. In addition, after fracture surgery, limb movement is restricted and the risk of muscle atrophy increases, which in turn raises the risk of joint wear and reinjury. Using organoid technology to culture and differentiate MSCs in vitro with osteogenic factors can effectively shorten the rehabilitation period and reduce the need for secondary surgeries, offering a new strategy for fracture treatment and bone defects[2]. Besides, the osteogenic differentiation of MSCs in vitro also holds significant scientific value for the study of calcification-related diseases. A research team from Shandong First Medical University investigated the molecular mechanisms underlying the development of calcific aortic valve disease by inducing

osteogenesis in human valvular interstitial cells in vitro[3]. This review elucidates the mechanisms by which they regulate the osteogenic differentiation of MSCs, as well as the facilitative effects of related activators observed during in vitro culture and differentiation. Studies have shown that mesenchymal progenitor cells in skeletal muscles near bones mediate initial fibrosis in damaged areas and participate in the formation of cartilage and bone, thereby playing a key role in bone regeneration; clinically, it has also been found that complete coverage by skeletal muscle can improve bone healing. Xing and others found that skeletal muscle is involved in chondrogenesis and endochondral ossification during the early stages of callus formation[4]. Irisin, a peptide fragment secreted synthetically by skeletal muscle, has also been shown to promote osteogenic differentiation, either exogenously or endogenously, by inhibiting lipogenic gene expression and activating the classical Wnt pathway[5]. Therefore, this review also includes the discussion of skeletal muscle directional differentiation.

The core process in the osteogenic differentiation of MSCs is osteogenesis. Osteogenesis is the process of bone tissue formation primarily carried out by osteoblasts. Mesenchymal cells, under the influence of growth factors, TGF-β, and signaling pathways, first differentiate into osteoprogenitor cells, and then further differentiate into osteoblasts. Osteoblasts migrate to bone formation sites and secrete bone matrix, constituting the process of bone formation[6].

2. Wnt Proteins and Corresponding Signaling Pathways

Wnt proteins are a class of highly conserved signaling proteins that play an important role in regulating development and modulating diseases such as cancer. In pluripotent MSCs, Wnts attach to the cell surface or extracellular matrix, and function through autocrine or paracrine mechanisms[7]. Figure 1 shows the three-dimensional structure of protein Wnt-5a, a typical factor in the family.

Figure 1: The three - dimensional structure of Protein Wnt-5a[8]

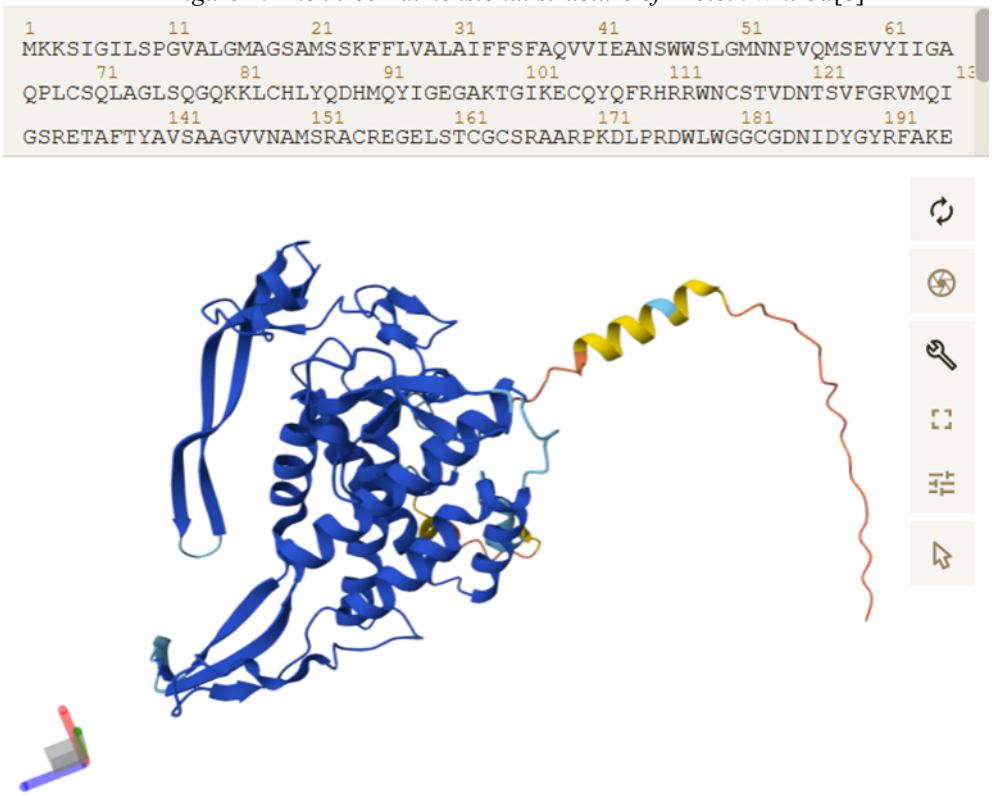
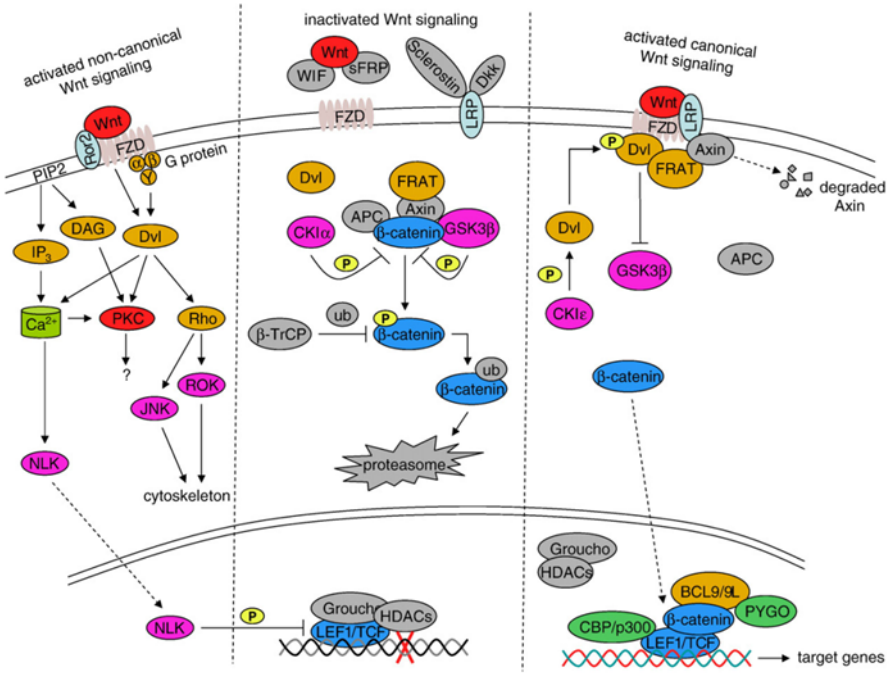


Figure 2: Schematic representations of the canonical and non - canonical Wnt pathway[7]



The Wnt signaling pathway is divided into canonical one and non-canonical one, with β-catenin serving as the hallmark protein of the canonical Wnt pathway. The canonical Wnt pathway is activated when Wnts bind to receptors Fzd and LRP5/6, resulting in decreased phosphorylation of β-catenin to prevent its binding modification by ubiquitin molecules and delivery to the proteasome, which then translocates into the nucleus to activate the transcription of specific genes (Figure 2).

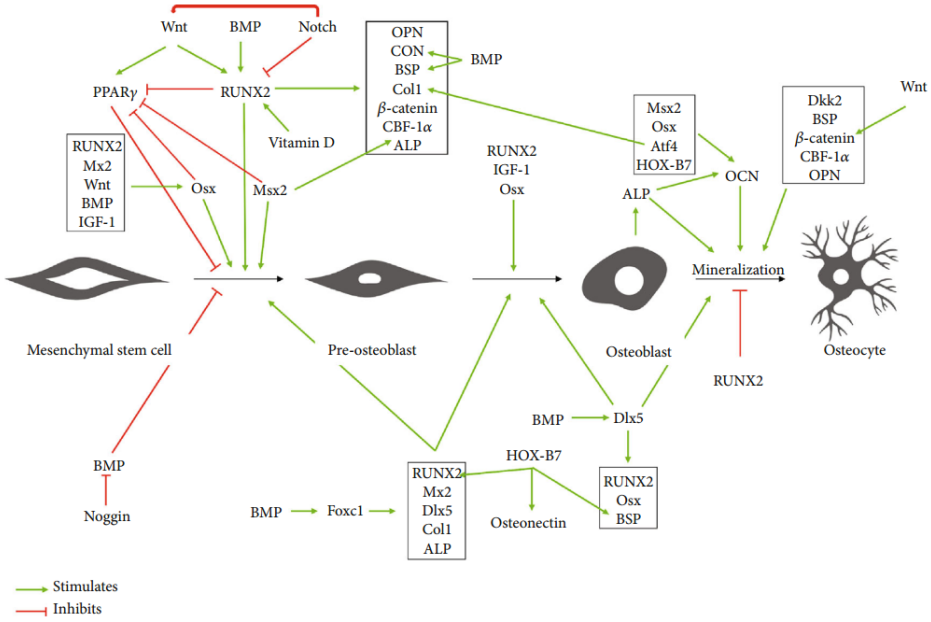
3. Osteogenesis-Related MSCs Differentiation

3.1 Osteogenic Differentiation

During the differentiation of osteoblasts, Wnt proteins influence cell fate by regulating the expression of a series of transcription factors, among which Runx2 and Osterix are two key transcription factors. Activation of the Wnt signaling pathway can promote the expression of Runx2 and Osterix, thereby facilitating the differentiation of osteoblasts into osteocytes and initiating the synthesis and deposition of bone matrix[6].

In the regular bone formation process, the impact of the canonical Wnt pathway on osteogenesis depends on the developmental stage of the target cells. The canonical pathway stimulates differentiation of MSCs while inhibiting the terminal differentiation of mature osteoblasts. Within the non-canonical signaling pathways, Wnt11 is upregulated during both osteogenic and chondrogenic differentiation of MSCs. Since non-canonical Wnt signaling is dynamically related to calcium ion signaling, promoting the osteogenic differentiation of osteoprogenitor cells by regulating the activation and expression of key transcription factors such as Runx2 and Osterix. In addition, by modulating calcium ion concentration, it can indirectly upregulate essential osteogenic genes like Runx2 and COL1A1, directly promoting bone matrix synthesis and mineralization[7]. Figure 3 displays effects of multiple bone differentiation-related biomolecules on various stages of osteogenic differentiation of MSCs.

Figure 3: The effects of bone differentiation-related biomolecules on the osteogenesis process[9]



3.2 Chondrogenic Differentiation

As for chondrogenesis, Wnt signaling exerts an inhibitory effect, corresponding to the upregulation of β-catenin in osteoprogenitor cells and its downstream regulation in chondroprogenitor cells. During this induction process, Sox9 is a specific transcription factor essential for chondrocyte differentiation and can suppress the transcriptional activity of Runx2 and β-catenin. The canonical Wnt pathway can regulate Sox9 to promote chondrogenic differentiation during the early stages of MSC differentiation. In the later stages, the antagonism between Sox9 and β-catenin has a significant effect[10].

3.3 Myogenesis

Wnt proteins serves as important stimulators of myogenesis in MSCs. The canonical Wnt pathway can induce myogenesis by activating the gene expression of myogenic regulatory factors (MRFs)[11]. Wnts directly affect myosatellite cells during the early stages of myogenesis. After the activation of Wnt, precursor cells shift from proliferation toward myogenic differentiation[12]. Conversely, inhibition of this pathway results in precursor cells differentiating toward adipocyte[13].

4. Critical Factors for In Vitro Culture and Differentiation of MSCs

4.1 In Vitro Differentiation of MSCs

At present, in vitro differentiation of MSCs is mainly used to construct in vitro models for providing medical strategies and tissue transplantation therapy[9]. The heterogeneity exhibited during the in vitro expansion and cultivation of MSCs leads to differences in cell morphology and physiological functions while the aging process is triggered, which affects the clinical outcomes of tissue transplantation. It has been found in research that specific culture conditions, such as hypoxic and low-temperature environments, are beneficial for MSCs to maintain their conformation and slow down the acceleration of aging caused by in vitro culture (Figure 4). Moreover, modifying and enhancing certain signaling pathways in MSCs also facilitates the expression of chemokines, thereby promoting the homing of MSCs to damaged areas for repair.

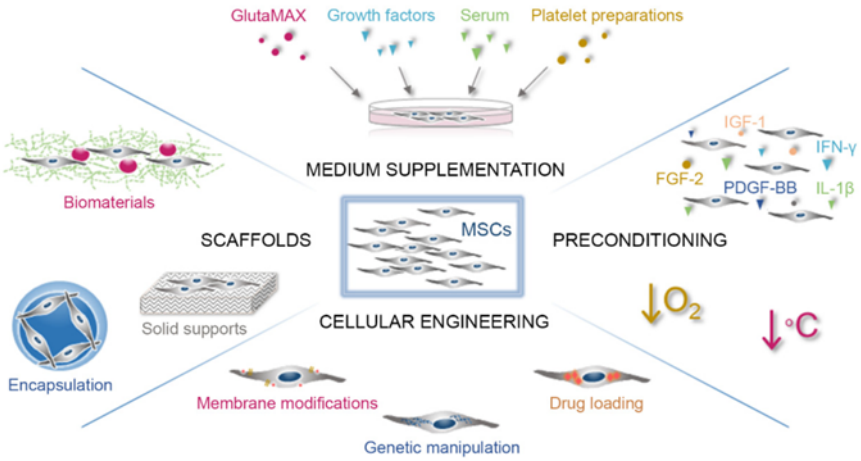


Figure 4 Various methods to promote the in vitro culture and differentiation of mesenchymal stem cells[14]

4.2 In Vitro Osteogenic Differentiation

In the laboratory, adding follicle-stimulating hormone β-subunit (FSHβ) to the culture medium can enhance the BMP9-induced transcription of cyclin D1,then augment BMP9-induced Wnt/β-catenin signaling, ultimately strengthening the osteogenic effect induced by BMP9[15].

4.3 In Vitro Chondrogenic Differentiation

Hypoxia-inducible factor (HIF-1α) can be added to the culture medium for in vitro chondrogenic differentiation. MSCs differentiate into chondrocytes in an avascular environment. HIF-1α functions to promote Sox9 gene expression under hypoxic conditions and simultaneously reduce protease-mediated degradation, thereby maintaining Sox9 mRNA levels, stimulating the expression of Sox5 and Sox6, and upregulating the expression of type II collagen and proteoglycans[16]. It has also been shown that HIF-1α has a role in co-regulating Inhibin β-A (INHBA), belonging to Sox9-independent genes, with HIF-2α to promote chondrogenic differentiation of MSCs under hypoxic conditions[17]. Another optional ingredient is lithium chloride (LiCl) .The effect of LiCl is to activate β-catenin, which promotes rapid proliferation of MSCs in the early stage and regulates the upregulation of Sox9 expression to enhance chondrogenic differentiation of MSCs. However, since β-catenin also promotes osteogenic differentiation, creating a strong antagonistic effect with Sox9 at a later stage of differentiation, excessive addition of LiCl causes MSCs to tend to differentiate into osteoblasts. Thus, the dosage of this inducer must be strictly controlled[18].

4.4 In Vitro Myogenic Differentiation

Researches have proved that Adipose-Derived Mesenchymal Stem Cells (ADSCs) have potential for myogenic differentiation both in vivo and in vitro [19]. It was found that the medium under low calcium concentration (calcium concentration <0.002 mmol/mL) promoted ADSC proliferation and differentiation in the direction of myogenesis. The potential mechanism may be the inhibitory effect of high calcium concentration on MyoG presence. Since the Wnt pathway exists to upregulate the expression of MRFs, the mechanism may be related to the inhibition of the Wnt pathway[20]. Through this process, MSCs differentiate into myosatellite cells, which differentiate into myofibroblasts and myoblasts in turn.

5. Discussion

In the in vitro culture of MSCs related to osteogenesis, although the proteins within the pathway and their interactions has already been proven for a long time, the effects of activators or inhibitors on the final

differentiation outcome have remained elusive due to the interactions between signaling pathways and the varying influences these pathways exert on MSCs at different stages of differentiation. At present, research methods on the effects of activators or inhibitors on in vitro differentiation remain limited to dosage titration, with feasibility judged solely by calcium deposition assays.

Since the Wnt signaling pathway relies on cellular transcription mechanisms, the author believes that chromatin immunoprecipitation followed by sequencing (ChIP-seq) technology can be used to study the specific binding sites of activators or inhibitors. ChIP-seq technology specifically enriches DNA fragments bound by target proteins through chromatin immunoprecipitation, enabling the identification of genomic loci and providing genome-wide information on interactions with histones, transcription factors, and other key regulators. In previous studies, researchers have used ChIP-seq technology to demonstrate that Sox transcription factors are essential for the formation of Wnt enhanceosomes by β-catenin, which itself lacks intrinsic DNA-binding activity, and for the recruitment of these complexes to different lineage-specific WNT-responsive enhancers (WREs), thereby regulating transcription[21]. The ChIP-seq technology also be applied to demonstrate that Sox transcription factors are essential for the formation of Wnt enhanceosomes by β-catenin, which itself lacks intrinsic DNA-binding activity, and for the recruitment of these complexes to different lineage-specific WREs, thereby regulating transcription[22]. It illustrates that ChIP-seq can more accurately determine the effects of added activators or inhibitors on transcription by pinpointing the binding sites of specific proteins or transcription factors.

There is a competitive relationship among the several differentiation pathways of MSCs, and the previously mentioned osteogenic and chondrogenic differentiation is a typical example. Osteoporosis, which is often seen in the elderly population, is also associated with a dysregulation of the competitive balance between the osteogenic and lipogenic directions of MSCs[23], which is often accompanied by lipid metabolism-related diseases[24]. ChIP-seq assist in exploring the interactions between differentiation directions by uncovering potential targets of activators or inhibitors in the transcriptional landscape, and provide new targets for disease-related drugs while improving the efficiency of in vitro differentiation. This provides new ideas and directions for the treatment of bone-related diseases.

6. Conclusion

As one of the key signaling pathways affecting cell differentiation, the Wnt pathway plays an extremely important role in the in vitro culture and differentiation of MSCs. This review starts by exploring the mechanisms through which the Wnt pathway affects MSCs, and discusses activators that can be utilized in the culture environment for osteogenic, chondrogenic, and myogenic differentiation. In addition, the review introduces the emerging ChIP-seq technology which holds significant value for identifying the specific transcriptional targets of signaling pathways involved in cell differentiation, assisting stem cells in osteogenesis-related directed differentiation cultures, shows the potential of application in tissue engineering techniques and establishment of in vitro models.

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