



FACTORS INFLUENCING OSTEOGENESIS

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The process of osteogenesis includes the well-coordinated proliferation and differentiation of osteogenic cells. It is known that mesenchymal stem cells are the progenitor cells in this process. They differentiate into osteoblasts and give rise to new bone formation. Osteogenesis happens not only as a normal process during embryonal development but also as result of some diseases, traumatic injury, and fractures. The mechanisms of physiological and pathological processes of ossification are similar. However, the factors participating have not yet been studied in detail. The involvement of genetic mechanisms, cell growth factors, pharmacological substances, biochemical pathways and nutrients are reported committing to osteogenic differentiation at multiple levels. In conclusion, osteogenesis is a complex process and a modern approach to the influencing factors is needed.

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INTRODUCTION

In order to review the current trends of the factors participating in osteogenesis, we pay attention to the factors influencing the differentiation of bone mesenchymal stem cells (BMSC), osteoblasts, osteoclasts and osteocytes. We point out the factors that have both inducing and suppressing effect on the process and mainly on the differentiating cells. We can classify those factors that commit to differentiation

at multiple levels, fine tuned with the involvement of genetic mechanisms, cell growth factors, pharmaceuticals and nutraceuticals (1), circadian rhythm and microenvironmental conditions (2). We also point out diseases, traumatic injuries and fractures like influencing factors because of the similarity of the mechanisms in the process of osteogenesis.

Most of the factors pointed above are in current research and their complete mechanisms of influencing the process of

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osteogenesis is yet to be investigated. The aim of this study is to provide the latest relevant information on influencing factors of the process of osteogenesis in both humans and rats. Current developments in osteogenesis and bone regeneration, including molecular and cellular mechanisms, physical therapies (low-level laser, distraction osteogenesis), biological therapies (mesenchymal stem cells, stem cell derived exosomes, inflammatory factor), as well as tissue engineering approaches promoting bone regeneration by targeting osteogenesis (3).

ASSESSMENT OF THE PROCESS OF OSTEOGENESIS

The process of bone formation is called osteogenesis or ossification. Osteogenesis is a core component of the skeletal system and depends on the well-coordinated proliferation and differentiation of osteogenic cells. Multiple signaling pathways and transcriptional factors tightly regulate this process. Any abnormalities in bone formation could cause severe disorders such as osteogenesis imperfecta and osteoporosis. Bone regeneration, a complex and well-orchestrated physiological process of osteogenesis, remains a medical challenge in the field of orthopedics and maxillofacial surgery. Based on its embryological origin, there are two types of ossification, called intramembranous ossification that occurs in mesenchymal cells that differentiate into osteoblast in the ossification center directly without prior cartilage formation and endochondral ossification in which bone tissue mineralization is formed through cartilage formation first. In intramembranous ossification, bone development occurs directly. In this process, mesenchymal cells proliferate into areas that have high vascularization in embryonic connective tissue in the formation of cell condensation or primary ossification centers. These cells will synthesize bone matrix in the periphery and the mesenchymal cells continue to differentiate into osteoblasts. After that, the bone will be reshaped and replaced by mature lamellar bone. Endochondral ossification will form the center of primary ossification, and the cartilage extends by proliferation of chondrocytes and deposition of cartilage matrix. After this formation, chondrocytes in the central region of the cartilage start to proceed with maturation into hypertrophic chondrocytes. After the primary ossification center is formed, the marrow cavity begins to expand towards the epiphysis. Then the subsequent stages of endochondral ossification will take place in several zones of the bone.

SIGNIFICANCE OF MSCS – ORIGIN OF BONE CELLS AND FUNCTION

The term “stem cell” can be applied to a diverse group of cells that share two characteristic properties: a capacity for prolonged or unlimited self-renewal under controlled conditions, and the potential to differentiate into a variety of specialized cell types. Mesenchymal stem cells (MSCs) are multipotent stromal cells with the ability to undergo self-renewal and multi-lineage differentiation (4). The term MSC was originally coined in reference to a hypothetical common progenitor of a wide range of “mesenchymal” (nonhematopoietic, nonepithelial, and mesodermal) tissues (5). The heterogeneous population of cultured plastic adherent cells isolated from the bone marrow, which remain the most commonly used (if not acknowledged) population by researchers in the field of bone regeneration, will be referred to as bone marrow stromal cells (BMSCs) (6). Adult mammalian bone marrow contains not one but two ostensibly discrete populations of adult stem cells. The first and by far the most fully characterized are the hematopoietic stem cells responsible for maintaining lifelong production of blood cells. The biological characteristics and properties of the second marrow resident population of stem cells, variously termed bone marrow stromal cells or mesenchymal stem cells, are in contrast much less well understood (7). Bone marrow mesenchymal stem cells (BMSCs) are readily available and possess a high osteogenic capacity. As a result, they are considered to be one of the most appropriate stem cell populations for bone regeneration and are thus widely used for efficiency comparison purposes with other cell sources. In a cell culture study the BMSCs demonstrate a typical spindle-shaped fibroblast-like morphology with a homogeneous shape and size with proliferative ability and gradual increase in the cell number over time (8). Several studies have demonstrated that BMSCs combined with suitable scaffolds are more effective in promoting new bone formation than scaffolds alone in animal bone defect models (9-11).

The function of BMSCs is crucial to the outcome of bone damage repair. After bone tissue injury, osteocytes sense mechanical changes and microdamage and initiate bone resorption, which is mediated by osteoclasts, and new bone generation, which is mediated by osteoblasts derived from BMSCs. Osteoclasts and osteoblasts jointly promote bone remodelling and microdamage repair. With increasing age, the number of BMSCs remains the same, whereas that of

mature osteoblasts decreases, suggesting that the osteogenic differentiation ability of BMSCs is impaired over time, leading to the failure of timely microdamage repair, bone loss, and an increased incidence of fractures. Improving the osteogenic differentiation of BMSCs in aged subjects with osteoporosis after fatigue loading may provide a new treatment strategy for bone microdamage repair and fracture prevention (12).

DIFFERENTIATION OF MSCS

Mesenchymal stem cells (MSCs) can become not only osteoblasts, but also adipocytes and chondrocytes, according to specific factors present in the microenvironment (13). MSCs can additionally exert a biologically supportive function through paracrine actions and trophic factors (14, 15).

To define MSCs in a common standard, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy has proposed the minimal criteria to define human MSCs (16). First, MSCs must be plastic-adherent when maintained in standard culture conditions. Second, MSCs must express CD105, CD73, and CD90, and lack expression of CD45, CD34, CD14, CD11b, CD79 α , CD19, and human leukocyte antigen-antigen D related (HLA-DR) surface molecules. Third, MSCs must differentiate to osteoblasts, adipocytes, and chondroblasts in vitro. As there

are no specific markers for MSCs, both positive markers and negative markers are applied to identify MSCs. The positive markers are expressed by MSCs, while negative markers should not be expressed by MSCs. Accordingly, in humans, Stro-1, CD29, CD44, CD73, CD90, CD105, CD146, and SSEA4 are positive combination markers for MSCs (17), while in mice, Sca-1, CD29, CD44, and CD105 are the positive markers (18). The differentiation toward the osteogenic lineage is tightly controlled by different molecular factors such as bone morphogenic proteins (BMPs) (19, 20) and wntless-related integration site (WNT) pathways (21, 22), but involves many other pathways, including that of nuclear factor κ B, which is typically considered important mostly for osteoclasts, as well as that of the NAD⁺-dependent deacetylase sirtuin-1 (23).

ORIGINS OF OSTEOBLAST AND FACTORS INFLUENCING OSTEOBLASTOGENESIS

Osteoblasts, bone-forming cells, arise from the commitment of mesenchymal precursors to osteoprogenitor lineages through the sequential action of transcriptional factors - RUNX2 and Osterix and β -Catenin being the most studied and found in both rat and human cells. Other factors include ATF4, AP1, a transcription factor composed of heterodimeric proteins of the Fos and Jun families (c-Jun, JunB et JunD; c-Fos, FosB, Fra-1, Fra-2) is an important regulator of bone

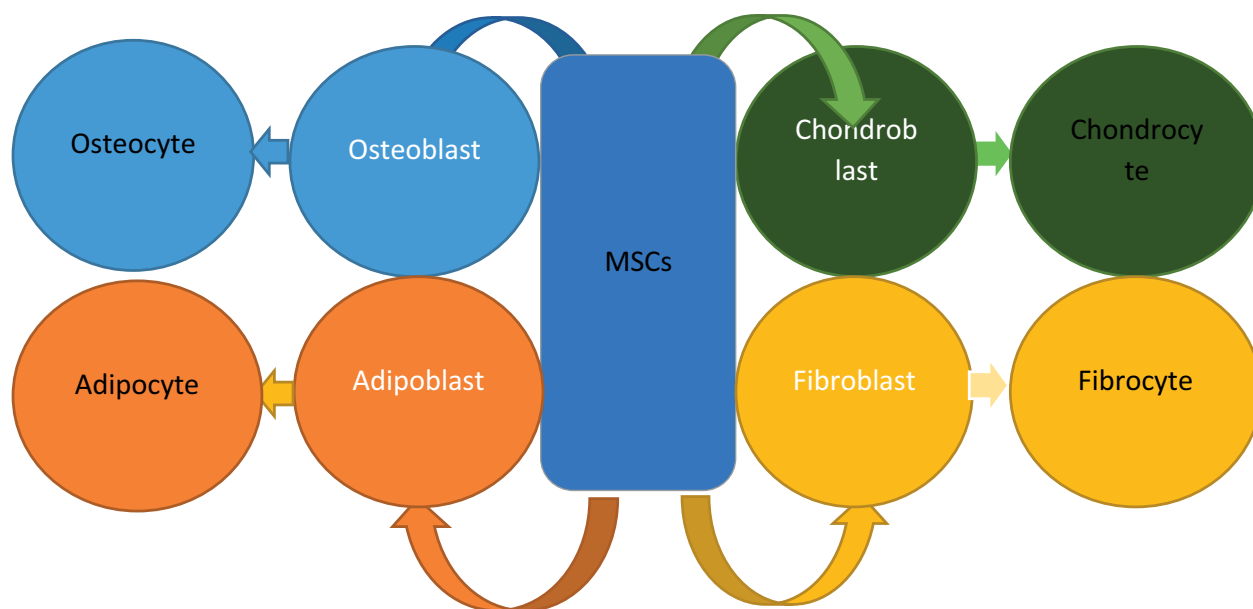


Figure 1. Lineage of differentiation of MSC cells. From: Kadreva (2023).

formation, as demonstrated by genetic manipulations in mice (24), Homeobox proteins (Msx1, Msx2, Dlx5, Dlx6), Helix–loop–helix (HLH) proteins, PPAR γ receptors. CCAAT/enhancer-binding proteins (C/EBPs) are transcription factors that regulate genes critical for adipocytic and osteoblastic differentiation (25). Most of the factors pointed above are in current research and their complete mechanisms of influencing the process of osteogenesis is yet to be discovered. However, transcription factors are microenvironmental factors and they can also be regulated by hormones such as parathyroid hormone (PTH), estrogens, glucocorticoids and vitamin D.

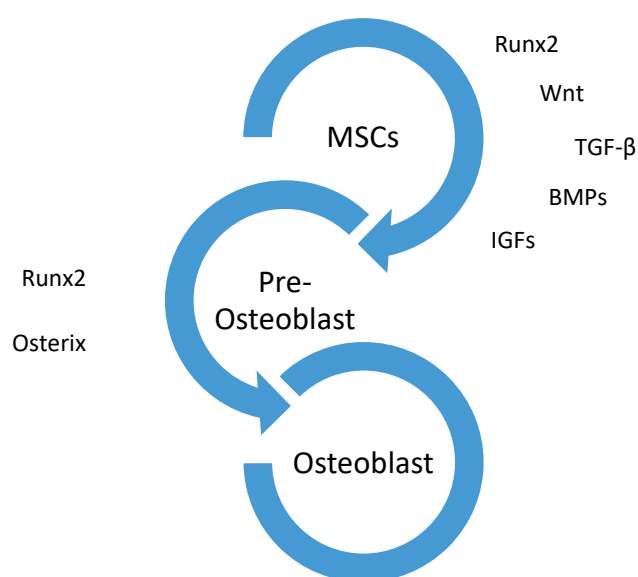


Figure 2. The process of osteoblastogenesis is tightly regulated by several common known factors shown in the figure above. Their activity is influenced by environmental factors, some imbalanced by known diseases such as osteoporosis etc., hormones, age and biochemical mechanisms. From: Kadreva (2023).

Mature osteoblasts produce extracellular proteins, including osteocalcin, alkaline phosphatase and type I collagen, the latter of which makes up over 90% of bone matrix protein. The extracellular matrix is first secreted as unmineralized osteoid and subsequently mineralized through the accumulation of calcium phosphate in the form of hydroxyapatite (26). This committed osteoprogenitor cell then becomes a pre-osteoblast, following the transcription of early osteogenic genes, such as alkaline phosphatase (ALP) and collagen1 α 1 chain (COL1A1), the expression of which is then kept throughout the mature osteoblasts' life, in addition to other common osteoblast

markers, such as osteopontin (OPN), bone sialoprotein II (BSP II) and osteocalcin (OCN). Once osteoblasts' synthetic activity is finished, they have three possible paths of differentiation: (1) undergoing apoptosis; (2) becoming bone lining cells, or (3) becoming osteocytes (27).

IMPORTANT FACTORS INFLUENCING OSTEOCLASTS

The differentiation process of osteoclasts is stimulated by a variety of signal molecules. However, the two most critical factors, M-CSF and RANKL, run through the entire process of osteoclast differentiation (28). Some studies have certified the indispensable role of M-CSF, in which the *M-csf* gene is mutated and expresses non-functional M-CSF protein in mice. As a result, these mice had fewer osteoclasts, leading to severe osteosclerosis (29). Purified osteocytes express RANKL more strongly than osteoblasts and support the formation of osteoclasts better, which indicates that osteocytes perform a vital function in the process of bone remodeling (30). Loss of RANKL in mice results in severe osteopetrosis, while overexpression of soluble RANKL leads to severe osteoporosis (31, 32). OPG, a member of the tumor necrosis factor (TNF) superfamily of proteins that is secreted by osteoblasts, is another key molecule in this process because it inhibits RANKL-induced osteoclastogenesis (33). In fact, OPG binds to RANKL with high affinity and competes with RANK for binding to RANKL on the surface of osteoclasts and their receptors (34). This RANK/RANKL/OPG system is regulated by various cytokines (interleukin-1, 4, 6, 11 and 17 and TNF- α), hormones (glucocorticoids, vitamin D and estrogen), and mesenchymal transcription factors (cbfa-1 and peroxisome proliferator-activated receptor gamma) and determines osteoclast activity. When the balance between bone resorption and formation is disrupted, bone diseases and disorders such as Paget's disease, fibrous dysplasia, osteoporosis and fragility fractures may occur. Recent advances have revealed new specific targets for the treatment of bone loss that are based on the inhibition of bone resorption by osteoclasts or the stimulation of bone formation by osteoblasts. A recent study reported of drugs, influencing different factors in bone cells such as Bisphosphonates, Selective Estrogen Receptor Modulators, Parathyroid Hormone Therapy, Strontium Ranelate and others (35).

Macrophage colony-stimulating factor (M-CSF) receptor has been previously reported to be present in osteoclasts both at mRNA and protein levels. Another study shows that I-labeled

M-CSF ligand specifically binds to the M-CSF receptor on osteoclasts by autoradiography; 2) binding of M-CSF to the receptor stimulates protein tyrosine phosphorylation in osteoclasts by immunostaining; 3) oxygen-derived free radicals produced by calvarial osteoclasts are increased by M-CSF stimulation (1.37 ± 0.08 , $n = 10$, $p < 0.01$); and (4) bone resorption in calvarial explants is enhanced by M-CSF (1.153 ± 0.09 , $n = 10$, $p < 0.001$) (36).

CONFLICT OF INTEREST

The authors state no conflict of interests.

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