Journal of Clinical and Medical Images

Review Article

ISSN: 2640-9615 |Volume 8

Molecular Signaling Pathways Governing Osteoblast Differentiation of Mesenchymal Stem Cells

Nihal Almuraikhi*

Stem Cell Unit, Department of Anatomy, College of Medicine, King Saud University, Riyadh 11461, Saudi Arabia

***Corresponding author:**

Nihal Almuraikhi, Stem Cell Unit, Department of Anatomy, College of Medicine, King Saud University, Riyadh 11461, Saudi Arabia

Received: 16 Dec 2024

Accepted: 31 Dec 2024

Published: 06 Jan 2025

J Short Name: JCMI

Citation:

Nihal Almuraikhi, Molecular Signaling Pathways Governing Osteoblast Differentiation of Mesenchymal Stem Cells. J Clin Med Img. 2025; V8(7): 1-8

1. Introduction

Adult human mesenchymal stem cells (MSCs) are of immense value in the regenerative medicine as they have potential to undergo self-renewal, produce trophic factors production and exhibit multilineage differentiation such as osteogenic, chondrogenic, adipogenic lineages [1,2]. These stem cells can be isolated from bone marrow, adipose tissue, dental tissues, dermal tissues, cord blood and various other tissues [2]. Although MSCs do not enjoy complete immune privilege, however, allogenic MSCs exhibit low immunogenicity with strong immunomodulation [2,3]. MSC– mediated immunomodulation is major histocompatibility complex (MHC) - independent and it is carried out by numerous paracrine factors, cytotoxic T lymphocytes (CD8+ T cells), natural killer (NK) cells and various other cells [3,4]. MSCs maintain homeostasis as they work as a sensor and switcher of the immune system i.e., they promote or suppress inflammatory processes when the immune system is underactive or overactive, respectively [5]. Owing to their self-renewal, low immunogenicity, and multilineage differentiation ability, MSCs offer a promising therapeutic approach in the field of regenerative medicine. One of the three criteria established by the International Society for Cellular Therapy (ISCT) for MSCs identification is their in vitro differentiation into osteoblasts, adipocytes, and chondrocytes [6]. In this context, the core functions of MSCs are immunoregulation and osteogenic differentiation, rendering them crucial cells for bone metabolism and immune system as there exist complex interactions between immune and skeletal systems [7]. On the one hand, T cells and B cells modulate the resorption and formation of bone by osteoclasts and osteoblasts respectively via a variety of cytokines [7]. On the other hand, MSCs regulate the T cell and B cell differentiation. The process of osteoblast differentiation of MSCs involves several intricate molecular pathways where a little aberration may result in bone-related disorders. This paper aims at reviewing the analysis of molecular pathways which control osteoblast differentiation of MSCs.

2. Molecular Pathways of Osteoblastic Differentiation of MSCs

Osteoblasts are derived from multipotent bone marrow MSCs capable of giving rise to multilineage cells [8]. This differentiation depends on the several molecular factors present in the microenvironment including proteins and pathways. Osteoblast differentiation of bone marrow MSCs is mediated through several signalling pathways including intracellular transcription factors (TFs), growth factors especially transforming growth factor beta (TGF-β), cytokines, and a variety of extracellular factors such as hypoxia, exercise and vibration, radiation, drugs, diet, and perivascular distribution of cells [8,9]. Molecular pathways involved in the osteoblast differentiation of MSCs include wingless-related integration site/beta-catenin (Wnt/β-catenin) pathway, bone morphogenic proteins (BMPs), Hedgehog signalling pathway, Notch signalling pathway, phosphatidylinositol 3-kinase/protein kinase B (PI3K-Akt) signalling pathway, JAK-STAT signalling pathway, microRNAs, Long Non-coding RNA, TGF-β signalling pathway, RANKL signalling pathway, ECM, and HIFs (Figure 1) [9-11].

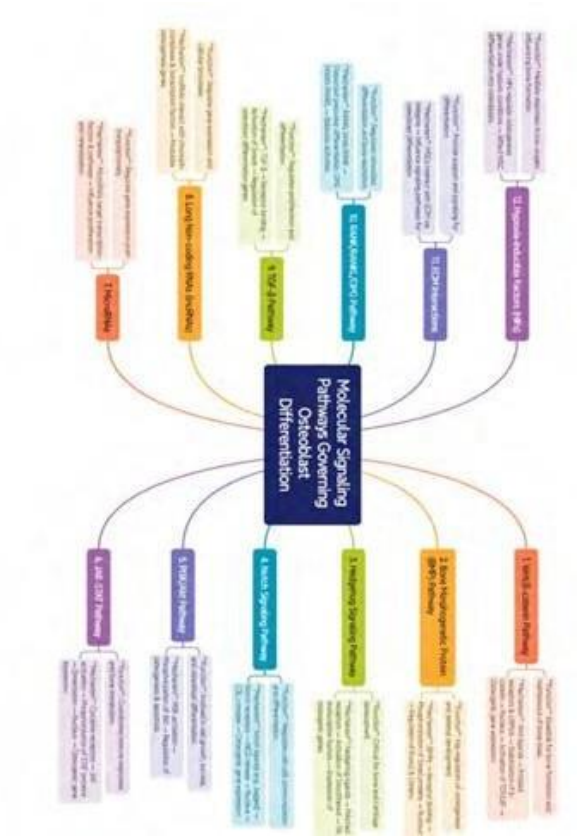

Figure 1: Molecular Signaling Pathways Governing Osteoblast Differentiation of Mesenchymal Stem Cells.

1. Wnt/β-catenin Signalling Pathway

The Wnt/β-catenin signalling plays a key role to maintain adult bone homeostasis and carry out skeletal development (e.g., organ development, injury repair, etc.) via cell determination, proliferation and polarity [12]. In this signalling pathway, Wnt ligands bind to the frizzled receptors and low-density lipoprotein receptor-related proteins 5 and 6 (LRP5/6) co-receptors, stabilizing β-catenin – one of the crucial TFs in Wnt/β-catenin signalling pathway [12,13]. Accumulated β-catenin moves into the nucleus and interacts with T-cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors to activate genes involved in osteoblast differentiation, bone formation and self-renewal [14].

Regarding bone regeneration, Wnt genes found in MSCs, osteoblasts and osteocytes, turn on canonical and non-canonical pathways which, in turn, enhance osteoblast differentiation of MSCs [15]. Wnt1 gene suppresses adipocyte differentiation of MSCs through canonical pathway, increasing the osteoblast differentiation. Wnt3A enhances osteoblast formation through both canonical and non-canonical pathways. Wnt4 contributes to osteoblast differentiation and hinders inflammation and remodeling via canonical pathway. Wnt5A enhances both osteoblast and osteoclast formation through activation and suppression of canonical pathway. Similarly, other members of Wnt gene family are involved in bone homeostasis. Therefore, hyperactivity or hypoactivity of this signalling pathway leads to sclerosteosis or osteoporosis, respectively [16].

Apart from bone homeostasis, Wnt/β-catenin signalling pathway is essential for adult tissue homeostasis. However, abnormal activation of this pathway leads to the development of various diseases of heart, lungs, liver, nervous system and metabolism, and tumours via pathological mechanisms involving cell differentiation, proliferation, inflammation and fibrosis [17]. Aberrant activation of the Wnt/β-catenin pathway results in various cardiovascular abnormalities such as congenital heart defects, cardiac hypertrophy, diabetic cardiomyopathy, arrhythmias, and fibrosis [18]. Dysregulation of Wnt/ β-catenin pathway also contributes to alcoholic liver disease, non-alcoholic fatty liver disease (ALD), (NAFLD), hepatocellular cancer (HCC), cholangiocarcinoma (CCA) and hepatoblastoma (HB) [19].

Imbalance of Wnt/β-catenin signalling pathway enhances metastasis of non-small cell lung cancer, pathogenesis of chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF), silicosis, bronchopulmonary dysplasia (BPD) and lung injuries associated with hyperoxia [14, 20]. Defective Wnt/β-catenin pathway is associated with neurodegenerative diseases e.g., Parkinson's disease (PD), Alzheimer's, ischemic stroke, Huntington's disease (HD), schizophrenia, and multiple sclerosis and amyotrophic lateral sclerosis – a class of motor neuron disease (MND) [21]. Similarly, dysfunction of this pathway paves way for the development of several metabolic disorders and cancers. Wnt ligands are involved in the processes essential for the development and progression of type 2 diabetes (T2DM) and its complications [22]. Therefore, dysregulation of the Wnt/β-catenin signalling pathway is also associated with the development of T2DM.

2. BMP and Smad-Dependant Signalling Pathway:

Along with multifunctional growth factors, bone morphogenetic proteins (BMPs) play a key role in osteoblast differentiation. Bone tissue is continuously destroyed and reformed via osteoclast (derived from hematopoietic precursors) and osteoblast (derived from mesenchymal cells) activity. Therefore, a precise balance is required between the osteoclast and osteoblast activities to maintain bone microarchitecture and function. Several growth factors contribute to bone homeostasis. BMPs belonging to the TGF-β superfamily play a pivotal role in bone formation [23]. Researchers have identified 12 different BMP ligands as well as they produced recombinant human BMPs (rhBMPs) – approved for therapeutic purposes especially in orthopaedic and dental applications [24]. For instance, rhBMP-2 is being used in the field of maxillofacial surgery as it works as a potent osteoinductive growth factor in bone formation [25]. Additionally, due to its osteogenetic potential, FDA has approved rhBMP-2 for tibial shaft repair and spinal

fusion surgery [26]. BMP signalling begins with the binding of BMP ligands to receptors on MSCs, resulting in phosphorylation and activation of Smad proteins. Activated Smads translocate to the nucleus where they regulate the transcription of osteogenic genes such as Runx2, Osterix and several other TFs [27]. Dysregulation of this pathway is associated with bone disorders in humans e.g., osteoporosis, enthesopathy, tooth decay, acromelic dysplasia, cardiospondylocarpofacial syndrome and Marfan syndrome [28-30]. Impaired BMP and Smad-dependant signalling pathway also paves way for several types of cancers e.g., HCC, colorectal cancer, breast cancer, renal cell carcinoma (RCC), ovarian cancer, glioma, and many others [31]. Similarly, dysregulation of BMP pathway results in various cardiovascular diseases e.g., pulmonary arterial hypertension (PAH), atherosclerosis, cardiac defects and hereditary hemorrhagic telangiectasia (HHT) [32].

3. Hedgehog Signalling Pathway:

Hedgehog (Hh) proteins play a key role in the regulation of musculoskeletal development and bone homeostasis [33]. This signalling pathway involves a series of Hh ligands, patched (Ptch) and smoothened (Smo) receptors, signalling regulators (e.g., SUFU) and transcription factors (glioma-associated oncogene) [34, 35]. Binding of Hh ligands to Ptch receptors relieves inhibition of Smo receptors, resulting in the activation of Gli TFs which, in turn, modulate the expression of target genes (i.e., osteogenic genes), thereby promoting osteogenic differentiation of stem cells [36].

Hedgehog signalling pathway plays a crucial role in the differentiation and proliferation of chondrocytes and osteocytes. Therefore, mutation in the genes involved in this signalling pathway or its dysregulation results in various genetic diseases e.g., holoprosencephaly (HPE), Greig cephalopolysyndactyly syndrome (GCPS), brachydactyly, acrocapitofemoral dysplasia, Joubert syndrome, Meckel syndrome, Pallister-Hall syndrome, osteoporosis, PD, Alzheimer's disease and many others [33, 37]. Bone-related diseases due to aberrations Hh signalling pathway include Werner's syndrome, acheiropodia, Laurin-Sandlow syndrome, polydactyly and syndactyly [38]. Additionally, aberrant activation of the Hh signalling pathway leads to neoplastic transformations, tumorigenesis (e.g., cancer of colon, liver, prostate, breast, ovary, brain and skin) and development of chemoresistance [39].

4. Notch Signalling Pathway:

Notch is a transmembrane receptor, and it mediates cell-cell interactions and regulates osteogenesis [40]. Interaction between Notch receptors (e.g., Notch1) and ligands (e.g., Jagged and Delta) triggers proteolytic cleavage to release Notch intracellular domain (NICD). Then, NICD travels to the nucleus and forms a transcriptional complex with CSL (CBF-1/suppressor of hairless/Lag1) and RBP-Jkappa (RBP-Jκ), promoting the expression of Hes and Hey family genes that influence osteoblast differentiation [40, 41]. Hence, the Notch signalling pathway potentially enhances osteoblast differentiation, promoting bone formation.

In a transgenic mouse model, osteoblast-specific overexpression of NICD results in osteosclerotic bone and mutations affecting Notch signalling via γ-secretase are associated with the development of age-related osteoporosis [41, 42]. In other words, aberrant Notch signalling leads to various cancerous and non-cancerous conditions. Notch signalling associated non-cancerous conditions which are caused by gene mutations include Alagille syndrome, spondylocostal dysostosis, Hajdu-Cheney disease, Adams-Oliver syndrome, bicuspid aortic valve disease, schizophrenia and left ventricular cardiomyopathy [41, 43]. Notch signalling associated non-cancerous conditions which are caused by factors other than gene mutations include PAH, non-alcoholic steatohepatitis (NASH), osteoarthritis (OA), graft versus host disease (GVHD), multiple sclerosis, pancreatitis, Duchene muscular atrophy, and Klippel Feil syndrome [41].

5. PI3K-Akt Signalling Pathway:

Phosphoinositide 3-kinase (PI3K) and Akt (protein kinase B) signalling pathways are also involved in osteoblast differentiation. Activation of PI3K leads to phosphorylation and activation of Akt, which regulates downstream targets involved in osteogenic gene expression, cell growth, proliferation and cell survival [44]. Dysregulation of the PI3K/Akt pathway leads to the occurrence of several conditions including cancer. For example, hyperactivation of this signalling pathway contributes to uncontrolled cell proliferation, evasion of apoptosis, and enhanced metastatic potential [45,46]. Dysregulation of this pathway leads to the development of osteoporosis [47], osteosarcoma [48] and Paget's disease of bone [49]. Additionally, hyperactive PI3K/Akt pathway leads to the development of atherosclerotic plaques and subsequent cardiovascular events [50]. Conversely, hypoactivation of the PI3K/Akt signalling pathway has been implicated in neurodegenerative disorders such as Alzheimer's disease [51].

6. JAK-STAT Signalling Pathway:

This signalling pathway works through Janus kinases, signal transducer and activator of transcription, cytokine receptors and several growth factors. Therefore, it is called as JAK-STAT signalling pathway that is involved in the bone homeostasis including regulation of osteoblasts and osteoclasts (differentiation and proliferation), tissue regeneration and angiogenesis in addition to modulation and migration of osteoclasts [52]. Activated Jaks phosphorylate STAT elements, leading to dimerization and translocation into the nucleus where STAT dimers regulate target genes involved in cellular processes. When it comes to the osteoblast differentiation of MSCs, activated STAT elements promote the expression of osteogenic genes e.g., Runx2, Osterix and osteocalcin [53]. Dysregulation in JAK-STAT signalling pathway induces cancers and immune diseases e.g., myeloproliferative neoplasms [54]. What about other pathways?

7. MicroRNAs:

As the name indicates, microRNAs (miRNAs) are small non-coding RNAs that regulate gene expression at the post-transcriptional level. Recently, miRNAs have emerged as key regulators of osteoblastic differentiation, influencing various aspects of this process MSCs. Several miRNAs have been identified as critical regulators of osteoblast differentiation as they affect proliferation, lineage commitment, and mineralization. The miR-29 family (miR-29a, miR-29b, and miR-29c) is known to suppress osteogenesis by targeting inhibitors of Wnt signalling and extracellular matrix components [55]. miR-34a has been identified as a negative regulator of osteoblast differentiation by targeting several osteogenic transcription factors, including Runx2 and Satb2 [56]. miR-133 inhibits osteoblast differentiation by targeting Runx2, one of the major regulators of osteogenesis [56]. miR-2861 is involved in promoting osteoblast differentiation by targeting histone deacetylase 5 (HDAC5), which suppresses Runx2 activity [57]. miR-21 is implicated in promoting osteoblast differentiation by targeting Smad7 – one of the potentially negative regulators of BMP signalling [58]. Specifically, upregulation of miR-21 enhances BMP signalling and stimulates osteogenesis. Dysregulation of miRNAs expression may alter biological processes which may lead to the occurrence of various diseases, cancers and acquisition of metastasis. As far as cancers are concerned, altered miRNAs expression has been reported in primary bone tumours e.g., osteosarcoma (OS), chondrosarcoma (CS) and Ewing's sarcoma [59, 60]. Similarly, aberrant miRNAs expression has also been identified in other musculoskeletal disorders including osteoporosis, osteoarthritis, rheumatoid arthritis and osteogenesis imperfecta [60, 61]. These diseases are usually attributed to the upregulation or downregulation of miRNAs. Deregulation of miRNAs has been reported in multiple myeloma (MM) [62]. Therefore, future therapeutics may target the inhibition or activation of certain miRNAs expression [60].

8. Long Non-Coding RNA

Long noncoding RNAs (lncRNAs) have gained significant attention in recent years as they are considered as a crucial layer of biological regulators in several processes, including regulation of gene expression and osteoblast differentiation from MSCs. Characteristically, lncRNAs are a class of transcripts having more than 200 nucleotides and devoid of protein-coding potential, however, they influence gene expression through diverse mechanisms such as chromatin remodeling, transcriptional regulation, and post-transcriptional modifications [63].

As far as osteoblast differentiation is concerned, lncRNAs play a key role they modulate the activity of key transcription factors and signalling pathways [64]. For instance, HOX transcript antisense RNA (HOTAIR) is a lncRNA that influences osteogenic differentiation by recruiting polycomb repressive complex 2 (PRC2) to specific gene loci, thus altering histone modification patterns and gene expression profiles associated with osteogenesis [65]. Moreover, interaction between lncRNAs miRNAs helps osteoblast differentiation of MSC, bone homeostasis and bone regeneration [66]. Dysregulation of lncRNAs (e.g., H19) leads to the development of various musculoskeletal disorders such as Li-Fraumeni syndrome - associated osteosarcoma, breast and lung cancers [67].

9. Transforming Growth Factor-Beta (TGF-β) Pathway

The TGF-β pathway is essential for osteoblast differentiation, a vital process in bone formation regulation cell proliferation in autocrine fashion [23]. TGF-β attaches to specific receptors on osteoprogenitor cells, provoking a series of intracellular signaling events primarily involving Smad proteins. Once activated, Smad2 and Smad3 form a complex with Smad4 and move into the nucleus, where they regulate the expression of key genes associated with osteoblast differentiation, including Runx2, Osterix, and collagen type I [68]. Additionally, TGF-β also interacts with other signaling pathways, such as Wnt and mitogen-activated protein kinase (MAPK), resulting in further osteoblast maturation and function. Disruption of the TGF-β pathway can result in poor bone healing and conditions like osteoporosis [69].

10. Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) Signalling Pathway

The RANKL pathway plays a crucial role in osteoblast differentiation and bone remodeling. Produced by osteoblasts and other cell types, RANKL binds to its receptor RANK on osteoclast precursors, promoting their maturation into mature osteoclasts, which are vital for bone resorption [70]. Simultaneously, RANKL also affects osteoblasts by enhancing their maturation and survival. When RANKL binds to RANK, it activates signaling pathways involving NF-κB and MAPK, which boost the expression of critical transcription factors like Runx2 and Osterix that drive osteoblast differentiation [71]. Disruption of the RANKL pathway can result in bone disorders such as osteoporosis, characterized by excessive bone resorption [71].

11. Extracellular Matrix (ECM) Interactions

Interactions with the ECM are essential for osteoblast differentiation and functionality. The ECM creates a dynamic microenvironment that shapes osteoblast behavior through various biochemical and mechanical signals. Key components like collagen, fibronectin, and laminin not only provide structural support but also actively participate in cell signaling. Integrins, which are receptors on the cell surface, facilitate the adhesion of osteoblasts to the ECM, initiating intracellular signaling pathways that drive osteogenic differentiation. For example, the interaction between integrins and ECM proteins activates focal adhesion kinase (FAK) and triggers subsequent pathways, including MAPK and Wnt signaling, which are crucial for expressing important osteogenic markers e.g., Runx2 and Osterix [72]. Disruptions in these ECM

interactions can hinder osteoblast function and contribute to bone diseases (e.g., osteoporosis), affecting the vital role of ECM in maintaining skeletal health and integrity [73].

12. Hypoxia-Induced Factors (HIFs)

The HIFs are crucial in regulating osteoblast differentiation in low-oxygen environments. Under hypoxic conditions, HIF-1α and HIF-2α become stabilized and move to the nucleus, where they turn on various target genes mediating osteogenesis [74]. These factors boost the transcription of key osteogenic markers, including Runx2, Osterix, and bone morphogenetic proteins (BMPs), thereby facilitating the differentiation of mesenchymal stem cells into osteoblasts [75]. The interaction between HIFs and other signaling pathways, such as Wnt and Notch, further refines the osteoblast differentiation process. Thus, disruption of HIF signaling can result in impaired bone development and healing, indicating the significance of hypoxia in skeletal biology.

3. Osteoblastic MSCs and Therapeutic Medicine

Owing to their multipotency, immunomodulation, broad secretory profile and self-renewal capacity, MSCs reap significant importance in regenerative medicine and therapeutics extending across several medical disciplines. Clinical trials and ongoing research aim at exploring applications of osteoblast MSCs in various conditions such as bone regeneration and repair, orthopaedic disorders and systemic disorders. Properties of MSCs like trans-differentiation, cell-fusion, mitochondrial transfer, release of microvesicles or exosomes indicate their potential for regenerative medicine and therapeutics [76]. Implications of MSCs in modern technologies like bioprinting, scaffolds and organoid models have shown promising outcomes [77]. In osteoporosis, bone formation is reduced, and fat accumulation is increased due to reduced capacity of MSCs to differentiate into osteoblasts and enhanced differentiation into adipocytes [78]. In other words, MSCs differentiation shifts from osteoblast to adipocytes, resulting in the development of osteoporosis. Current treatment options for osteoporosis are based on the drug-based agents that suppress bone resorption and enhance bone anabolism e.g., bisphosphonates, denosumab, teriparatide and romosozumab [78]. With the advent of cell-based therapy, MSCs have garnered a focus on osteoporosis treatment. In this context, both allogenic and autologous MSCs transplantation has shown promising outcomes in animal models [79]. Recently, Amasoud et al. [80] studied and reported that tankyrase inhibitor XAV-939 is a powerful up-regulator of osteoblast differentiation of human MSCs (hMSCs) that stipulates that it can be an effective therapeutic modality to treat the conditions associated with low bone formation such as osteoporosis. Tankyrase, a polymerase, regulates Wnt signalling pathway and subsequent osteoblast differentiation of MSCs [80]. AlMuraiki et al. [81] studied Fedratinib, a JAK2 inhibitor, and reported that it blocks the differentiation of hMSC-TERT cells into osteoblasts. Therefore, the Food and Drug Authority (FDA) has approved Fedratinib for the treatment of myelofibrosis – a myeloproliferative neoplasm [82]. In another study, Al-Muraikhi et al. [83] identified ruxolitinib as a JAK-STAT pathway inhibitor leading to the inhibition of osteoblast differentiation and matrix mineralization of MSCs. Hence, it is evident that inhibitors of JAK-STAT pathway may be considered in future for the therapeutic purposes against the conditions caused by aggravated osteoblast differentiation and mineralization. Similarly, inhibition of Hh signalling pathway with Smoothened antagonist has shown reduced osteoblast differentiation of hMSCs and ectopic bone formation – maybe a novel therapeutic option for conditions with hyperactive osteoblast activities and ectopic bone formation [84]. In a study, the researchers identified a potent γ-secretase and Notch signalling inhibitor (LY411575) which led to the suppression of processes involved in the osteoblast differentiation of hMSCs [85]. Therefore, Notch pathway inhibitors can be implicated in therapeutic efforts against the conditions with hyperactive osteoblast differentiation of MSCs e.g., Kaposi's sarcoma, MM, glioblastoma and many other cancers [86]. However, one thing that must not be ignored is that γ-secretase inhibitor regulates both osteoblast and osteoclast differentiation, warranting a precise and extremely careful implication of this agent in regenerative medicine and therapeutics.

4. Conclusion

Osteoblastic differentiation of MSCs is an extremely regulated process controlled by different molecular pathways that interact in a well-coordinated manner to enhance bone formation. Therefore, dysregulation or disruption of these pathways may result in skeletal disorders. In future, trials on the elucidation of the intricate molecular mechanisms governing osteoblast differentiation will pave the way for novel therapeutic approaches aimed at enhancing bone regeneration and treating various bone diseases.

References

1. Jovic D, Yu Y, Wang D, Wang K, Li H, et al. A brief overview of global trends in MSC-based cell therapy. Stem Cell Rev Rep. 2022;18(5): 525-45.
2. Li Z, Hu X, Zhong JF. Mesenchymal stem cells: characteristics, function, and application. Stem cells Int. 2019; 8106818.
3. Weiss AR, Dahlke MH. Immunomodulation by mesenchymal stem cells (MSCs): mechanisms of action of living, apoptotic, and dead MSCs. Front Immunol. 2019; 10: 1191.
4. Kadri N, Amu S, Iacobaeus E, Boberg E, Le Blanc K. Current perspectives on mesenchymal stromal cell therapy for graft versus host disease. Cell Mol Immunol. 2023; 20(6): 613-25.
5. Jiang W, Xu J. Immune modulation by mesenchymal stem cells. Cell Prolif. 2020; 53(1): e12712.
6. Hernández R, Jiménez-Luna C, Perales-Adán J, Perazzoli G, Melguizo C, Prados J. Differentiation of human mesenchymal stem cells towards neuronal lineage: clinical trials in nervous system disorders. Biomol Ther. 2020; 28(1): 34-44.

7. Ye F, Li J, Xu P, Xie Z, Zheng G, Liu W, et al. Osteogenic differentiation of mesenchymal stem cells promotes c-Jun-dependent secretion of interleukin 8 and mediates the migration and differentiation of CD4+ T cells. Stem Cell Res Ther. 2022; 13(1): 58.
8. Vasiliadis AV, Galanis N. Human bone marrow-derived mesenchymal stem cells from different bone sources: a panorama. Stem Cell Invest. 2020; 7: 15.
9. Gao Q, Wang L, Wang S, Huang B, Jing Y, Su J. Bone marrow mesenchymal stromal cells: identification, classification, and differentiation. Front Cell Dev Biol. 2022; 9: 787118.
10. Ponzetti M, Rucci N. Osteoblast differentiation and signalling: established concepts and emerging topics. Int J Mol Sci 2021;22(13):6651.
11. Almuraikhi N. Inhibition of TGF-β type I receptor by SB505124 down-regulates osteoblast differentiation and mineralization of human mesenchymal stem cells. Cell Bioch Funct 2023;41(5):564-72.
12. Silva-García O, Valdez-Alarcón JJ, Baizabal-Aguirre VM. Wnt/β-catenin signaling as a molecular target by pathogenic bacteria. Front Immunol 2019; 10:2135.
13. Ren Q, Chen J, Liu Y. LRP5 and LRP6 in Wnt signaling: similarity and divergence. Front Cell Dev Biol. 2021; 9:670960.
14. Liu J, Xiao Q, Xiao J, Niu C, Li Y, Zhang X, et al. Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic opportunities. Signal Transduct Target Ther. 2022;7(1):3.
15. Vlashi R, Zhang X, Wu M, Chen G. Wnt signaling: Essential roles in osteoblast differentiation, bone metabolism and therapeutic implications for bone and skeletal disorders. Genes Dis. 2022;10(4):1291-317.
16. Kim JH, Liu X, Wang J, Chen X, Zhang H, Kim SH, et al. Wnt signaling in bone formation and its therapeutic potential for bone diseases. Ther Adv Musculoskelet Dis. 2013;5(1):13-31.
17. Jridi I, Canté-Barrett K, Pike-Overzet K, Staal FJ. Inflammation and Wnt signaling: target for immunomodulatory therapy? Front Cell Dev Biol. 2021; 8:615131.
18. Ni B, Sun M, Zhao J, Wang J, Cao Z. The role of β-catenin in cardiac diseases. Front Pharmacol. 2023; 14:1157043.
19. Zou G, Park JI. Wnt signaling in liver regeneration, disease, and cancer. Clin Mol Hepatol. 2023;29(1):33.
20. Liu M, Huo Y, Cheng Y. Mechanistic regulation of Wnt pathway-related progression of chronic obstructive pulmonary disease airway lesions. Int J Chron Obst Pulmon Dis. 2023; 18:871-80.
21. Anand AA, Khan M, V M, Kar D. The Molecular Basis of Wnt/β-Catenin Signaling Pathways in Neurodegenerative Diseases. Int J Cell Biol. 2023:9296092.
22. Nie X, Wei X, Ma H, Fan L, Chen WD. The complex role of Wnt ligands in type 2 diabetes mellitus and related complications. J Cell Mol Med. 2021;25(14):6479-95.
23. Lademann F, Hofbauer LC, Rauner M. The bone morphogenetic protein pathway: the osteoclastic perspective. Front Cell Dev Biol. 2020; 8:586031.
24. Lowery JW, Rosen V. Bone morphogenetic protein–based therapeutic approaches. Cold Spring Harb Perspect Biol. 2018;10(4): a022327.
25. On SW, Park SY, Yi SM, Park IY, Byun SH, Yang BE. Current status of recombinant human bone morphogenetic protein-2 (rhBMP-2) in maxillofacial surgery: should it be continued? Bioengineering. 2023;10(9):1005.
26. Halloran D, Durbano HW, Nohe A. Bone morphogenetic protein-2 in development and bone homeostasis. J Dev Biol. 2020;8(3):19.
27. Zhu S, Chen W, Masson A, Li YP. Cell signaling and transcriptional regulation of osteoblast lineage commitment, differentiation, bone formation, and homeostasis. Cell Discovery. 2024;10(1):71.
28. Zou ML, Chen ZH, Teng YY, Liu SY, Jia Y, Zhang KW, et al. The Smad dependent TGF-β and BMP signaling pathway in bone remodeling and therapies. Front Mol Biosci. 2021; 8:593310.
29. Liu M, Goldman G, MacDougall M, Chen S. BMP signaling pathway in dentin development and diseases. Cells. 2022;11(14):2216.
30. Costantini A, Guasto A, Cormier-Daire V. TGF-β and BMP signaling pathways in skeletal dysplasia with short and tall stature. Ann Rev Genomics Hum Genet. 2023;24(1):225-53.
31. Ehata S, Miyazono K. Bone morphogenetic protein signaling in cancer; some topics in the recent 10 years. Front Cell Dev Biol. 2022; 10:883523.
32. Ye D, Liu Y, Pan H, Feng Y, Lu X, Gan L, et al. Insights into bone morphogenetic proteins in cardiovascular diseases. Front Pharmacol. 2023; 4:1125642.
33. Lu W, Zheng C, Zhang H, Cheng P, Miao S, Wang H, He T, et al. Hedgehog signaling regulates bone homeostasis through orchestrating osteoclast differentiation and osteoclast–osteoblast coupling. Cell Mol Life Sci.2023;80(6):171.
34. Zhou H, Zhang L, Chen Y, Zhu CH, Chen FM, Li A. Research progress on the hedgehog signalling pathway in regulating bone formation and homeostasis. Cell Prolif.2022;55(1): e13162.
35. Sigafoos AN, Paradise BD, Fernandez-Zapico ME. Hedgehog/GLI signaling pathway: transduction, regulation, and implications for disease. Cancers. 2021;13(14):3410.
36. Ahmadi A, Mazloomnejad R, Kasravi M, Gholamine B, Bahrami S, Sarzaeem MM, et al. Recent advances on small molecules in osteogenic differentiation of stem cells and the underlying signaling pathways. Stem Cell Res Ther. 2022;13(1):518.
37. Sasai N, Toriyama M, Kondo T. Hedgehog signal and genetic disorders. Front Genet. 2019; 10:1103.
38. Onodera S, Azuma T. Hedgehog-related mutation causes bone malformations with or without hereditary gene mutations. Int J Mol Sci. 2023;24(16):12903.
39. Sari IN, Phi LT, Jun N, Wijaya YT, Lee S, Kwon HY. Hedgehog signaling in cancer: a prospective therapeutic target for eradicating cancer stem cells. Cells. 2018;7(11):208.
40. Lin GL, Hankenson KD. Integration of BMP, Wnt, and notch signaling pathways in osteoblast differentiation. J Cell Biochem. 2012;112(12):3491-501.

41. Zhou B, Lin W, Long Y, Yang Y, Zhang H, Wu K, et al. Notch signaling pathway: architecture, disease, and therapeutics. Signal Transduct Target Ther. 2022;7(1):95.
42. Liang ST, Chen JR, Tsai JJ, Lai YH, Hsiao CD. Overexpression of notch signaling induces hyperosteogeny in zebrafish. Int J Mol Sci. 2019;20(15):3613.
43. Zieba JT, Chen YT, Lee BH, Bae Y. Notch signaling in skeletal development, homeostasis and pathogenesis. Biomolecules. 2020;10(2):332.
44. Ramazzotti G, Ratti S, Fiume R, Follo MY, Billi AM, Rusciano I, et al. Phosphoinositide 3 kinase signaling in human stem cells from reprogramming to differentiation: a tale in cytoplasmic and nuclear compartments. Int J Mol Sci. 2019;20(8):2026.
45. Bahar ME, Kim HJ, Kim DR. Targeting the RAS/RAF/MAPK pathway for cancer therapy: from mechanism to clinical studies. Signal Transduct Target Ther. 2023;8(1):455.
46. Luo J, Manning BD, Cantley LC. Targeting the PI3K-Akt pathway in human cancer: rationale and promise. Cancer Cell. 2003;4(4):257-62.
47. Xi JC, Zang HY, Guo LX, Xue HB, Liu XD, Bai YB, et al. The PI3K/AKT cell signaling pathway is involved in regulation of osteoporosis. J Recep Sig Transd. 2015;35(6):640-5.
48. Xiang Y, Yang Y, Liu J, Yang X. Functional role of MicroRNA/PI3K/AKT axis in osteosarcoma. Front Oncol. 2023; 13:1219211.
49. Fukuda K, Funakoshi T. Metastatic extramammary Paget's disease: pathogenesis and novel therapeutic approach. Front Oncol. 2018; 8:38.
50. Zhao Y, Qian Y, Sun Z, Shen X, Cai Y, Li L, et al. Role of PI3K in the progression and regression of atherosclerosis. Front Pharmacol. 2021; 12:632378.
51. Razani E, Sigaroodi AP, Azar AS, Zoghi A, Bavarsad MS, Bashash D. The PI3K/Akt signaling axis in Alzheimer's disease: a valuable target to stimulate or suppress? Cell Stress Chaperones. 2021;26(6):871-87.
52. Wang P, Zhang Z. Bone marrow-derived mesenchymal stem cells promote healing of rabbit tibial fractures via JAK-STAT signaling pathway. Exp Ther Med. 2020;19(3):2310-6.
53. Chan WC, Tan Z, To MK, Chan D. Regulation and role of transcription factors in osteogenesis. Int J Mol Sci. 2021;22(11):5445.
54. Hu X, Li J, Fu M, Zhao X, Wang W. The JAK/STAT signaling pathway: from bench to clinic. Signal Transduct Target Ther. 2021;6(1):402.
55. Horita M, Farquharson C, Stephen LA. The role of miR-29 family in disease. J Cell Biochem. 2021;122(7):696-715.
56. Mazziotta C, Lanzillotti C, Iaquinta MR, Taraballi F, Torreggiani E, Rotondo JC, et al. MicroRNAs modulate signaling pathways in osteogenic differentiation of mesenchymal stem cells. Int J Mol Sci. 2021;22(5):2362.
57. Choe N, Shin S, Joung H, Ryu J, Kim YK, Ahn Y, et al. The microRNA miR-134-5p induces calcium deposition by inhibiting histone deacetylase 5 in vascular smooth muscle cells. J Cell Mol Med. 2020;24(18):10542-50.
58. Garcia J, Delany AM. MicroRNAs regulating TGFβ and BMP signaling in the osteoblast lineage. Bone. 2021; 143:115791.
59. Scuderi SA, Calabrese G, Paterniti I, Campolo M, Lanza M, Capra AP, et al. The biological function of MicroRNAs in bone tumors. Int J Mol Sci. 2022;23(4):2348.
60. Yu L, Li W, Yang P, Zhang W, Tao H, Ge G, et al. Osteoblastic microRNAs in skeletal diseases: biological functions and therapeutic implications. Eng Regen. 2022;3(3):241-57.
61. Guery AM, Millet M, Merle B, Collet C, Bagouet F, Borel O, et al. Dysregulation of microRNAs in adult osteogenesis imperfecta: the miROI study. J Bone Miner Res. 2023;38(11):1665-78.
62. Chen D, Yang X, Liu M, Zhang Z, Xing E. Roles of miRNA dysregulation in the pathogenesis of multiple myeloma. Cancer Gene Ther. 2021;28(12):1256-68.
63. Wang L, Qi L. The role and mechanism of long non-coding RNA H19 in stem cell osteogenic differentiation. Mol Med. 2021; 27:1-6.
64. Aurilia C, Donati S, Palmini G, Miglietta F, Iantomasi T, Brandi ML. The involvement of long non-coding RNAs in bone. Int J Mol Sci. 2021;22(8):3909.
65. Li Y, Sun W, Li J, Du R, Xing W, Yuan X, et al. HuR-mediated nucleocytoplasmic translocation of HOTAIR relieves its inhibition of osteogenic differentiation and promotes bone formation. Bone Res. 2023;11(1):53.
66. Lanzillotti C, De Mattei M, Mazziotta C, Taraballi F, Rotondo JC, Tognon M, et al. Long non-coding RNAs and microRNAs interplay in osteogenic differentiation of mesenchymal stem cells. Front Cell Dev Biol. 2021; 9:646032.
67. Liao J, Chen B, Zhu Z, Du C, Gao S, Zhao G, et al. Long noncoding RNA (lncRNA) H19: An essential developmental regulator with expanding roles in cancer, stem cell differentiation, and metabolic diseases. Genes Dis. 2023;10(4):1351-66.
68. Lu X, Li W, Wang H, Cao M, Jin Z. The role of the Smad2/3/4 signaling pathway in osteogenic differentiation regulation by ClC-3 chloride channels in MC3T3-E1 cells. J Orthop Surg Res. 2022;17(1):338.
69. Wu M, Wu S, Chen W, Li YP. The roles and regulatory mechanisms of TGF-β and BMP signaling in bone and cartilage development, homeostasis and disease. Cell Res. 2024;34(2):101-23.
70. Takegahara N, Kim H, Choi Y. RANKL biology. Bone. 2022; 159:116353.
71. Wu Z, Li W, Jiang K, Lin Z, Qian C, Wu M, et al. Regulation of bone homeostasis: signaling pathways and therapeutic targets. Med Comm. 2024;5(8): e657.
72. Liu Z, Wang Q, Zhang J, Qi S, Duan Y, Li C. The mechanotransduction signaling pathways in the regulation of osteogenesis. Int J Mol Sci. 2023;24(18):14326.
73. Licini C, Lin X. Changes in extracellular matrix associated with bone disorders. Front Endocrinol .2024; 15:1386459.
74. Mendoza SV, Genetos DC, Yellowley CE. Hypoxia Inducible Factor 2α Signaling in the Skeletal System. J Bone Miner Res Plus. 2023;7(4): e10733.

75. Qin Q, Liu Y, Yang Z, Aimaijiang M, Ma R, Yang Y, et al. Hypoxia-inducible factors signaling in osteogenesis and skeletal repair. Int J Mol Sci. 2022;23(19):11201.
76. Vasanthan J, Gurusamy N, Rajasingh S, Sigamani V, Kirankumar S, Thomas EL, et al. Role of human mesenchymal stem cells in regenerative therapy. Cells. 2020;10(1):54.
77. Ma Y, Deng B, He R, Huang P. Advancements of 3D bioprinting in regenerative medicine: Exploring cell sources for organ fabrication. Heliyon. 2024;10(3): e24593.
78. Hu L, Yin C, Zhao F, Ali A, Ma J, Qian A. Mesenchymal stem cells: cell fate decision to osteoblast or adipocyte and application in osteoporosis treatment. Int J Mol Sci. 2018;19(2):360.
79. Jiang Y, Zhang P, Zhang X, Lv L, Zhou Y. Advances in mesenchymal stem cell transplantation for the treatment of osteoporosis. Cell Prolif. 2021;54(1): e12956.
80. Almasoud N, Binhamdan S, Younis G, Alaskar H, Alotaibi A, Manikandan M, et al. Tankyrase inhibitor XAV-939 enhances osteoblastogenesis and mineralization of human skeletal (mesenchymal) stem cells. Sci Rep. 2020;10(1):16746.
81. AlMuraikhi N, Alaskar H, Binhamdan S, Alotaibi A, Kassem M, Alfayez M. JAK2 inhibition by fedratinib reduces osteoblast differentiation and mineralisation of human mesenchymal stem cells. Molecules. 2021;26(3):606.
82. Saleh K, Ribrag V. An evaluation of fedratinib for adult patients with newly diagnosed and previously treated myelofibrosis. Expert Rev Hematol. 2023;16(4):227-36.
83. AlMuraikhi N, Ali D, Alshanwani A, Vishnubalaji R, Manikandan M, Atteya M, et al. Stem cell library screen identified ruxolitinib as regulator of osteoblastic differentiation of human skeletal stem cells. Stem Cell Res Ther. 2018;9(1):319.
84. AlMuraikhi N, Almasoud N, Binhamdan S, Younis G, Ali D, Manikandan M, et al. Hedgehog signaling inhibition by smoothened antagonist BMS-833923 reduces osteoblast differentiation and ectopic bone formation of human skeletal (Mesenchymal) stem cells. Stem Cell Int. 2019;(1):3435901.
85. AlMuraikhi N, Ali D, Vishnubalaji R, Manikandan M, Atteya M, Siyal A, et al. Notch signaling inhibition by LY411575 attenuates osteoblast differentiation and decreased ectopic bone formation capacity of human skeletal (mesenchymal) stem cells. Stem cell Int. 2019;(1):3041262.
86. Song C, Zhang J, Xu C, Gao M, Li N, Geng Q. The critical role of γ-secretase and its inhibitors in cancer and cancer therapeutics. Int J Biologic Sci. 2023;19(16):5089.