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Review Article

# Transcriptomic signatures of osteogenic differentiation in human mesenchymal stem cells: A systematic review transcriptomics of osteogenic MSC differentiation

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## Abstract

**Background:** The osteogenic differentiation of human mesenchymal stem cells (hMSCs) is foundational for bone tissue engineering. Transcriptional profiling has been carried out to characterize important genes and pathways determining osteoblast lineage commitment.

**Purpose of Study:** To synthesise recent transcriptomic evidence from 2020-2025 describing gene expression signature, active signalling pathways, cellular heterogeneity, and non-coding RNA regulation associated with the osteogenic differentiation of human MSCs.

**Methodology:** A structured search of relevant literature was made in PubMed and Scopus to identify peer-reviewed articles that used techniques of bulk RNA sequencing and single cell sequencing to analyse osteogenically induced human MSC cells.

**Results:** Overall, across all studies, the process of osteogenic differentiation was consistently linked to activation of the evolutionarily conserved transcription and expression profile centred on RUNX2, as well as upregulation of extracellular matrix-related genes and important signalling pathways for osteogenic differentiation, including Wnt/β-catenin and BMP/TGF-β pathways. Additionally, single-cell RNA studies suggest heterogeneity and transitional differentiation states across single-cell populations, indicating an asynchronous and lineage-primed process for osteogenic differentiation. Moreover, a significant regulatory effect was highlighted for non- coding RNAs, including microRNAs, circular RNAs, and long non-coding RNAs, on the osteogenic expression profile.

**Conclusion:** In recent studies of the transcriptome related to hMSCs, the overall molecular mechanism for the differentiation of these stem cells in bone tissue has been revealed to be conserved but dependent upon the tissue from where the MSCs were obtained and the analytical technique used for the determination of the differentiation potential of these stem cells.

**Keywords:** RNA sequencing, Transcriptomic markers, Osteogenic lineage, Mesenchymal stem cells, and Bone regeneration

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## 1. Introduction

Given that human mesenchymal stem cells can differentiate into osteoblasts, they are extremely important for orthopaedic and regenerative medicine applications. Transcriptomic technology advancements, mainly RNA sequencing (RNA-seq) and single-cell RNA sequencing (scRNA-seq), have provided gene expression changes with respect to MSC osteogenic differentiation. With recent transcriptomic studies, some work has aimed to characterize conserved and context-specific gene signatures, providing information on regulatory pathways and cellular heterogeneity. In view of the growing number of transcriptome-based studies, a systematic synthesis is needed to consolidate those results to isolate molecular signatures that may be repeated.

## 2. Methodology

### *2.1. Purpose of study*

The objectives of this systematic review are to systematically collect and integrate recent studies on human stem cell transcriptomics to identify conserved and source-specific molecular regulators of human mesenchymal stem cells' osteogenic differentiation, with focuses on recent findings regarding genetic expression profiles, signalling pathways, cellular heterogeneity, and non-coding RNA roles.

### *2.2. Search strategy*

The systematic review is conducted based on the Preferred Reporting Items for Systemic Review and Meta-Analysis (PRISMA) standards.

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**Table 1:** Features of included transcriptomic research on human MSC osteogenic differentiation (2020–2025)

| Author (Year) | MSC source | Transcriptomic method | Osteogenic induction duration | Key findings |
|---|---|---|---|---|
| Pan et al.,[1] | Human Bone Marrow MSCs | Bulk RNA-Seq | 0-25 days | RUNX2- centered regulatory network; activation of ossification genes |
| Zhou Y et al.,[2] | Human Maxillary Sinus MSCs | Bulk RNA-Seq | 21 days | Upregulation of BMP5, IGF1, JUNB, SMOC2 during osteogenesis |
| Meesuk L et al.,[3] | Human Umbilical Cord MSCs | Small RNA-Seq and miRNA inhibition | 28 days | miRNA inhibition enhanced osteogenic gene expression (RUNX2, COL1A1) |
| Qu R et al.,[4] | Human Adipose - Derived MSCs | Single-cell RNA-Seq | 14 and 21 days | Identification of transitional osteogenic subpopulations and heterogeneity |
| Yang L et al.,[5] | Human Bone Marrow MSCs | RT-qPCR and functional validation study supporting transcriptomic pathway findings | 2 weeks | Wnt7a activation promotes RUNX2 expression and osteogenic differentiation |

An extensive literature survey has been carried out through various studies published between January 2020 and December 2025 based on the relevant keywords listed below. “Human mesenchymal stem cells,” “Osteogenic differentiation,” and “RNA sequencing,” “Transcriptome,” “Single-cell sequencing.”

### *2.3. Eligibility criteria*

The review has selected studies which meet following inclusion criteria:

1. Investigated human mesenchymal stem cells that were osteogenically induced *in vitro*.
2. Utilised bulk RNA sequencing (RNA-seq) methodologies or single-cell RNA sequencing (scRNA-seq).
3. Observed differentially expressed genes, transcriptomes, or signalling pathway profiles associated with osteogenic differentiation.

Studies were excluded if:

1. Were review articles, conference abstracts.
2. Animal models or Non-human cells used.
3. Focused on non-transcriptomic methodologies.
4. No osteogenic differentiation outcomes.

### *2.4. Study Selection and Data Synthesis*

The process of selecting studies was carried out using a PRISMA approach. The databases used for database searching were PubMed and Scopus, and a total of 132 results were retrieved. This number was then reduced to 94 after removing 38 duplicate articles and excluding another 82 articles based on a title and abstract screening not meeting the predetermined criteria. The full-text screening revealed 12 articles, but five studies met all the set criteria and thus were deemed eligible for qualitative synthesis. Further, peer-reviewed papers were cited but only to complement the context of cellular osteogenic signal pathways, regulation of non-coding RNAs, and single cell transcriptome studies. These were not part of the initial inclusion criteria and were made only for support.

Given the variability of MSC sources, sequencing technologies applied, cell differentiation paradigms, and analysis types, a narrative synthesis of findings has been applied instead of a meta- analysis. Similarly, findings have been synthesized on a theme based level of genes expressed, pathways engaged, single cell types, and non-coding RNAs regulation.

## 3. Results

The findings in regard to the already conducted studies’ respective transcriptomics are presented in a hierarchical manner, beginning with gene-level signatures’ conservation in various cell types and organisms to next include findings regarding pathways’ level of various kinds of regulating mechanisms, findings in regard to single-cell level analysis results, and last but not least, non-coding RNA.

### *3.1. Overview of included research*

The included studies employed MSCs from a range of human tissues, including bone marrow, adipose tissue, umbilical cord, and maxillary sinus membranes, demonstrating variability in cell origin and differentiation capacity.[1–5] This diversity in MSC tissue origin and transcriptomic methodology provides a foundation for interpreting both conserved and context-specific osteogenic gene expression patterns. **(Table 1)**

**Table 2:** Important widely expressed genes and pathways in hmsc osteogenic differentiation

| Gene/Pathway | Regulation | Functional role | Supporting References |
|---|---|---|---|
| RUNX | Upregulated | Master osteogenic transcription factor | Pan et al.,[1] Yang L et al.,[5] |
| BMP5 | Upregulated | Bone morphogenesis and mineralization | Zhou Y et al.,[2] |
| IGF1/IGF2 | Upregulated | Cell proliferation and extracellular matrix synthesis | Zhou Y et al.,[2] |
| JUNB | Upregulated | Osteoblast maturation | Zhou Y et al.,[2] |
| SMOC2 | Upregulated | ECM and mineralization regulation | Zhou Y et al.,[2] |
| Wnt/β-catenin pathway | Upregulated | Regulation of osteogenic gene transcription | Yang L et al.,[5] |

### *3.2. Conserved osteogenesis of gene signatures*

Throughout osteogenic differentiation, a subset of genes remains persistently overexpressed, reflecting a conserved transcriptional program underlying bone formation. Among these, RUNX2 has been identified as a central regulatory hub that orchestrates downstream osteogenic gene expression, as demonstrated by transcriptome network analyses of human bone marrow derived mesenchymal stem cells (MSCs).[1] In addition to RUNX2 centred regulation, RNA sequencing studies of human maxillary sinus derived MSCs have revealed significant upregulation of genes involved in bone morphogenesis, growth factor signalling, and extracellular matrix organization, including BMP5, IGF1, IGF2, JUNB, and SMOC2.[2]

Transcriptome analyses performed under diverse osteogenic culture conditions further indicate that differentiation is associated with the coordinated activation of gene networks related to skeletal development and extracellular matrix assembly.[6] Consistent with this observation, multiple studies have reported sustained upregulation of extracellular matrix associated genes such as COL1A1, COL1A2, SPARC, and BGLAP during osteogenic differentiation. Bulk RNA sequencing of bone marrow derived MSCs demonstrates a progressive increase in the expression of these matrix-related genes during mineralization, corresponding to osteoblast maturation and extracellular matrix organization.[6] These results together reinforce the notion that the expression of extracellular matrix gene activation is a core and universal transcriptional characteristic of osteogenesis found in all MSC sources. Though this gene expression signature captures an overlapping osteogenic core program, pathway level investigations provide deeper evidence for the synchronized regulatory processes for osteogenic differentiation.

### *3.3. Enrichment of signalling pathways and regulatory networks*

Aside from gene expression change, rich signalling pathways mediating osteogenic transcriptional programs have been previously explored. Of these, Wnt/β-catenin signalling consistently is upregulated within the context of mesenchymal stem cell (MSC) differentiation, with functional data indicating Wnt7a enhances RUNX2 transcription and promotes osteogenic differentiation in human MSCs. By contrast, studies highlighting extracellular matrix gene expression during phases of differentiated mineralization have identified more prominent BMP/TGF-β signalling, potentially attributing its specific role toward the matrix deposition and maturation stages of osteogenesis.[2]

An integrated RNA sequencing analysis of human induced pluripotent stem cell lines MSCs identified dynamic changes of transcription factor networks regulating osteogenic gene expression. These networks comprise canonical regulators, including RUNX2, among other transcriptional modulators recognized by co expressing analyses.[7] Indeed, one of the transcriptomics studies has revealed the existence of relevant regulatory genes which are in turn actively responsible for continuing osteogenic differentiation while maintaining an ongoing regulatory mechanism.[1] Although PI3K/Akt signalling was not investigated explicitly in some of these analyses, enrichment of this pathway has been reported in other transcriptomic studies of MSC osteogenesis, where it seems to promote cell survival and increase differentiation efficiency during longer term osteogenic induction. Taken together, this evidence reveals the interrelated role of multiple signalling pathways in orchestrating osteogenic differentiation, as demonstrated in the table of differentially expressed genes and pathways in human MSCs. **(Table 2)**

### *3.4. Single cell transcriptomics views*

While bulk RNA sequencing captures global trends in gene expression, single-cell RNA sequencing (scRNA-seq) provides critical insight into cellular heterogeneity during osteogenic differentiation. A scRNA-seq study of human adipose-derived mesenchymal stem cells identified distinct cellular subpopulations and transitional transcriptional states, demonstrating that osteogenesis proceeds through asynchronous and heterogeneous trajectories rather than a uniform differentiation process.[4] Although further functional validation is required, these findings suggest that resolving mesenchymal stem cell heterogeneity at single cell resolution may enable more precise molecular targeting strategies.

Unlike those based on bulk transcriptomics, single cell studies show early lineage primed subpopulations concealed by population-level reports to clarify the contribution of cellular heterogeneity to osteogenic transcriptional programs.[4] Consistent with this finding, in independent scRNA-seq studies of human bone marrow-derived mesenchymal stem cells, diverse but functionally distinct subpopulations with different immunoregulatory abilities and osteogenic potential were detected.[8] Taken as a whole, the studies reveal that intrinsic heterogeneity among the mesenchymal stem cell populations favours differential osteogenic capability and that particular subpopulations most likely play a disproportionately critical role in driving osteogenesis.

### *3.5. Non-coding RNA integration role*

In addition to protein coding genes, non-coding RNAs (ncRNAs) play crucial roles in regulating osteogenic differentiation. Multiple microRNAs (miRNAs) modulate osteogenic gene expression; for example, inhibition of miR-21, miR-29a, and members of the let-7 family (let-7a, let-7b, let-7c, etc.) enhances osteogenesis in human umbilical cord derived mesenchymal stem cells (MSCs), accompanied by increased expression of RUNX2 and collagen associated genes. Beyond miRNAs, circular RNAs (circRNAs) such as circ-CTTN show dynamic expression patterns and promote osteogenesis when upregulated, revealing an additional layer of post- transcriptional regulation.[9,10] Similarly, long non-coding RNAs (lncRNAs), including MALAT1 and H19, act in concert with miRNAs and transcription factors to activate RUNX2 and support osteogenic differentiation.[11] Both miRNAs and lncRNAs have been shown to regulate key osteogenic signaling pathways, including Wnt/β-catenin and TGF-β/BMP, influencing lineage commitment and differentiation outcomes.[12]

Transcriptomic profiling shows that during MSC osteogenesis, ncRNAs establish dense interaction networks with messenger RNAs (mRNAs). The presence of differentially expressed lncRNAs in the transcription can be accompanied by parallel changes in miRNA and mRNA, suggesting coordinated regulatory cascades governing osteogenic lineage commitment. Enrichment of these ncRNA–mRNA modules in processes important for osteogenesis, including proliferation, organization of the extracellular matrix, differentiation of osteoblasts, wound healing, and hypoxia response, has been identified through network analyses of high-throughput sequencing data. KEGG pathway analysis highlights additional regulatory nodes influenced by ncRNAs, including PI3K-Akt, Wnt, and calcium signalling pathways. Collectively, these insights indicate the involvement of a complex transcriptional-post-transcriptional cascade orchestrated by transcriptional and post-transcriptional mechanisms at the multidimensional regulatory network in MSC osteogenesis.

## 4. Discussion

In this systematic review, we show that osteogenic differentiation of human MSCs reveals a conserved transcriptome core that involves RUNX2 activation and coordinated regulation of growth factors, extracellular matrix genes, and signalling pathways. Although bulk RNA-seq studies yield strong identification of dominant osteogenic genes, single-cell transcriptomics demonstrate considerable heterogeneity and intermediate differentiation states. Between study comparisons imply MSC tissue origin influences the type and extent of transcriptomic responses, contributing to variability in osteogenic capacity of the tested subjects. The non-coding RNA analyses are also well integrated, indicating the multi-layered regulation of osteogenesis.[13]

At the gene, pathway level, RUNX2 consistently appears as the key transcriptional regulator governing osteogenic commitment across all sources of MSCs. Upstream signal pathways, for instance the Wnt/β-catenin pathway and the BMP/TGF-beta pathway, synergize in their action on RUNX2 to induce osteoblast fate commitment, while downstream induction of extracellular matrix-related genes reflects progress in cell maturation[14] of particular interest, MSC cells derived from sources in the craniofacial complex, for instance, maxillary sinus, displayed upregulation in some osteogenesis-related genes, e.g., IGF1 and JUNB, indicating potential tissue- specific transcriptional adaptation, which could have implications for osteogenesis efficacy.

Though bulk RNA sequencing has been shown to produce significant insight into general trends in transcriptional regulation in given systems, single-cell sequencing has shown that osteogenic differentiation is an asynchronous and heterogeneous process. It has been shown to result from lineage-primed populations and transitional transcriptional intermediates transitions that cannot be accounted for via bulk methods and may, as has been evidenced, result in heterogeneous and disparate osteogenic capabilities observed in various studies to date.

Besides the osteogenic-related protein coding genes, the involvement of non-coding RNAs has provided further evidence for an additional layer of complexity in the control of osteogenic differentiation pathways. Besides microRNAs and their involvement in the control of osteogenic transcription factors by these microRNAs targeting the RUNX2 transcription factors and their related pathways, the contribution of circular RNAs and long non-coding RNAs has provided evidence for the significance of these molecules in the control of the osteogenic transcription networks.[11] Even if the PI3/Akt pathway has not been studied specifically for its involvement in osteogenesis, the enriched pathway has been identified in related transcriptomic analyses for the control of the survival of mesenchymal stem cells during their osteogenic differentiation pathways. Taken together, the current data suggest that the regulatory mechanisms involved in the osteogenic differentiation of human MSCs are complex and involve the regulation at multiple levels, with conserved transcriptional regulation, the intrinsic cellular variation within the population itself, tissue specificity, and regulation through ncRNAs. Future studies involving the combination of these approaches will be important to improve the potential of the molecular signatures involved for bone tissue engineering and medicine.[15]

## 5. Conclusion

Transcriptomic studies consistently identify RUNX2-driven gene networks and Wnt/β- catenin signalling as the main character in the process of osteogenic differentiation in human MSCs, according to transcriptomic investigations between 2020–2025. Differences in gene expression patterns are due to MSC source and analytical approach variation.

On that note, osteogenic molecular signatures should be further refined for translational applications with future studies combining bulk and single-cell transcriptomics and functional validation that complement traditional methods.

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