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The Role of RUNX2 as a Master Regulator of Osteoblast Differentiation: A Narrative Review

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ABSTRACT

Runt-related transcription factor 2 (RUNX2) is a central transcriptional regulator of osteoblast-lineage commitment and bone formation. This narrative review synthesizes evidence on the stage-dependent functions of RUNX2, the signaling and molecular mechanisms that regulate its expression and activity, its downstream osteogenic gene network, and its relevance to bone regeneration. Fifteen original experimental studies were compared, supported by review articles and selected clinical literature and mechanistic literature. The evidence supports a major role for RUNX2 in early osteogenic commitment and indicates that RUNX2 also contributes to bone matrix gene expression and postnatal skeletal maintenance in committed osteoblasts. Its regulation involves BMP/SMAD, Wnt/β-catenin, MAPK, and PI3K/AKT-associated signaling, as well as non-coding RNAs, chromatin modification, lactylation, and ubiquitination-dependent protein stability. Human evidence from cleidocranial dysplasia further links pathogenic RUNX2 variants with characteristic skeletal and dental abnormalities. Nevertheless, RUNX2 expression alone cannot establish complete osteoblast maturation or functional matrix mineralization. Differences in cell models, differentiation stages, experimental stimuli, and measurement methods complicate comparisons among studies.

Keywords: RUNX2; osteoblast differentiation; osteogenesis; cleidocranial dysplasia; osteogenic markers; bone tissue engineering

INTRODUCTION

Bone formation and regeneration depend on the coordinated differentiation of mesenchymal progenitor cells into osteoblast-lineage cells. These cells produce the extracellular matrix and support its subsequent mineralization. This process is controlled by interacting signaling pathways and transcription factors that regulate osteogenic gene expression [1,2].

Among these regulators, runt-related transcription factor 2 (RUNX2), also known as core-binding factor subunit alpha-1 (CBFA1), is a key determinant of osteoblast-lineage commitment. RUNX2 belongs to the RUNX transcription factor family and contains a conserved Runt domain that mediates sequence-specific DNA binding. Its interaction with core-binding factor beta (CBFβ) enhances DNA-binding capacity and contributes to transcriptional regulation [2,21].

RUNX2 coordinates the expression of genes involved in osteoblast differentiation and bone matrix formation. These include SP7, COL1A1, COL1A2, BGLAP, SPP1, and IBSP, although the strength of evidence for direct transcriptional regulation differs among individual genes. Their products contribute to lineage progression, collagen-rich matrix formation, matrix organization, and mineralization [2,3,21]. Experimental studies using conditional Runx2 deletion indicate that RUNX2 also contributes to bone matrix gene expression in committed osteoblasts [3,22].

Despite this central role, RUNX2 expression is not a direct or comprehensive measure of osteoblast maturation. RUNX2 expression and activity vary with differentiation stage, while mRNA abundance, protein concentration, nuclear localization, DNA binding, and transcriptional activity represent distinct biological measurements [3,7,21].

In biomaterial research, this distinction is important because increased RUNX2 expression alone cannot establish functional matrix mineralization. This review discusses the biological role of RUNX2, its stage-dependent functions, upstream signaling pathways, post-transcriptional and epigenetic regulation, downstream osteogenic markers, methods used to evaluate RUNX2, human genetic evidence from cleidocranial dysplasia, and its relevance to scaffold-based bone regeneration.

METHODS

Literature was identified primarily through PubMed/MEDLINE, with Scopus, Web of Science, ScienceDirect, SpringerLink, and Google Scholar used as supplementary sources. The literature search focused on RUNX2 and its role in osteoblast differentiation, including its molecular regulation, associated signaling pathways, downstream osteogenic markers, and relevance to bone tissue engineering. Search terms included RUNX2, CBFA1, osteoblast differentiation, osteogenic differentiation, BMP/SMAD signaling, TGF-β signaling, Wnt/β-catenin signaling, MAPK/ERK signaling, PI3K/AKT signaling, osteogenic markers, cleidocranial dysplasia, and bone tissue engineering.

Publications from 2021 to 2025 were prioritized, while foundational studies were retained when necessary to explain established biological concepts. No formal language or full-text availability filters were documented. Fifteen original experimental studies were selected for detailed comparison, alongside eleven additional supporting publications, comprising eight review articles, two earlier foundational experimental studies, and one clinical genetic study. The selected primary studies were examined for their experimental models, interventions, RUNX2 assessment methods, sampling times, additional osteogenic markers, functional outcomes, and principal findings. As the initial exploratory searches were not recorded, the exact number of records retrieved, screened, and assessed in full text could not be retrospectively established.

Studies were considered relevant when they (i) investigated RUNX2 expression, protein abundance, localization, transcriptional activity, or RUNX2-dependent osteogenic outcomes; (ii) examined signaling or molecular mechanisms linked to osteoblast-lineage differentiation; or (iii) provided human genetic or clinical evidence directly relevant to RUNX2. Original experimental studies published mainly from 2021 to 2025 were prioritized for the 15-study comparison matrix. Review articles were used to contextualize mechanisms, while selected earlier foundational studies were retained when they addressed a key unresolved biological question. Studies were not included in the primary comparison matrix when they were reviews, did not directly examine RUNX2-related osteogenic biology, or were used only to provide clinical or historical context. The selection was purposive rather than exhaustive; therefore, selection bias is possible.

Information was extracted from the full texts of the 15 selected original studies, including abstracts, Materials and Methods, Results, figure legends, tables, and supplementary information where available. Extracted items included study design, cell and animal models, experimental interventions and concentrations, RUNX2 assay type and sampling time, additional osteogenic markers, functional outcomes, and principal findings. Evidence was organized thematically and compared according to the biological endpoint actually measured. “Not assessed” indicates that an endpoint was not measured; “NR” is reserved for details not reported by the article. No formal screening flow, duplicate independent screening, or risk-of-bias assessment was undertaken.

RESULTS AND DISCUSSION

1. Biological characteristics and essential role of RUNX2

RUNX2 is a transcription factor in the RUNX family, which also includes RUNX1 and RUNX3. Its conserved Runt domain mediates binding to specific DNA sequences, while interaction with CBFβ enhances DNA-binding capacity and supports RUNX2 protein function. Through these properties, RUNX2 regulates transcriptional programs associated with osteoblast-lineage commitment and bone matrix formation [2,5].

Experimental evidence supports an essential role for RUNX2 in osteoblast development and bone formation. Qin et al. [3] used osteoblast-specific Runx2 deletion in mice and reported reduced trabecular and cortical bone parameters, decreased bone formation, and lower expression of major bone matrix genes, including Col1a1, Col1a2, Spp1, Ibsp, and Bglap/Bglap2. Deletion of Runx2 in primary osteoblasts also impaired osteoblast differentiation and matrix-protein gene expression. These findings support a continuing contribution of RUNX2 after osteoblast-lineage commitment, in addition to its established role during early lineage specification.

RUNX2 abundance is also controlled after transcription. Dong et al. [4] reported that farnesoid X receptor (FXR) promotes osteogenic activity by suppressing Thoc6-mediated RUNX2 ubiquitination, thereby increasing RUNX2 protein stability. This finding illustrates that RUNX2 protein abundance may be regulated through post-translational mechanisms in addition to changes in gene expression.

Nevertheless, RUNX2 should not be considered the sole determinant of osteoblast identity. Its activity is coordinated with other transcription factors, particularly SP7/Osterix, and with extracellular signaling pathways. RUNX2 is therefore essential for normal osteoblast development, but its expression alone is insufficient to establish a fully differentiated osteoblast phenotype [2,5].

1.1. Human genetic evidence: cleidocranial dysplasia

Cleidocranial dysplasia (CCD) is a human skeletal and dental disorder strongly associated with pathogenic variants in RUNX2. Its clinical features include delayed closure of cranial sutures and fontanelles, hypoplastic or aplastic clavicles, and dental abnormalities such as supernumerary teeth and delayed or failed eruption of permanent teeth. These manifestations provide clinically relevant evidence that disruption of RUNX2 affects both skeletal development and odontogenesis. In a series of five unrelated patients and their families, Thaweesapphithak et al. [24] identified RUNX2 variants and documented variable dental, craniofacial, and osseous phenotypes, including multiple unerupted permanent teeth. A 2024 systematic review analyzing 453 individuals further described the broad phenotypic spectrum associated with RUNX2 variants and reported genotype–phenotype associations involving the Runt homology domain [25]. The clinical variability cautions against assuming a one-to-one relationship between a particular variant and a fixed phenotype. For dental medicine, CCD is especially relevant because delayed eruption, supernumerary teeth, and craniofacial abnormalities may require coordinated radiographic, orthodontic, surgical, and genetic assessment [24,25].

2. Stage-dependent functions of RUNX2 during osteoblast differentiation

Osteoblast differentiation progresses from mesenchymal progenitor commitment through osteoprogenitor and preosteoblast stages, followed by osteoblast maturation and matrix mineralization. RUNX2 has a prominent role during early osteogenic commitment, when progenitor cells acquire an osteoblast-lineage phenotype. It also promotes the expression of downstream transcription factors, including SP7, which contribute to subsequent differentiation [2,5].

The role of RUNX2 is not restricted to early lineage commitment. Qin et al. [3] showed that RUNX2 remains necessary in committed osteoblasts because its deletion reduces the expression of COL1A1, COL1A2, SPP1, IBSP, and BGLAP. This finding supports a continuing role for RUNX2 in regulating bone matrix-associated genes after osteoblast-lineage commitment.

RUNX2 expression and activity should be interpreted in relation to differentiation stage. Stage dependence does not mean that RUNX2 follows one universal pattern of continuous decline: its functions include lineage commitment, osteoprogenitor proliferation, induction of SP7, and regulation of matrix-protein genes in immature osteoblasts [21]. Earlier literature proposed that excessive or sustained RUNX2 activity may interfere with aspects of terminal maturation [5], but this should not be generalized to all models or interpreted as evidence that RUNX2 becomes dispensable after commitment. Conditional deletion studies and their differing outcomes indicate that the effect of RUNX2 loss depends partly on the genetic strategy and the functional protein produced [3,21,22]. Therefore, increased RUNX2 expression at one time point is not, by itself, proof of greater osteoblast maturity.

This stage-dependent interpretation is also relevant when comparing experimental studies. Some studies measure early transcriptional responses, whereas others evaluate later matrix production or mineral deposition. For example, Bayram et al. [18] measured RUNX2 mRNA on day 7, BMP2 on day 14, and OCN on day 21, illustrating that the timing of marker assessment can influence the biological interpretation.

3. Molecular signaling pathways regulating RUNX2

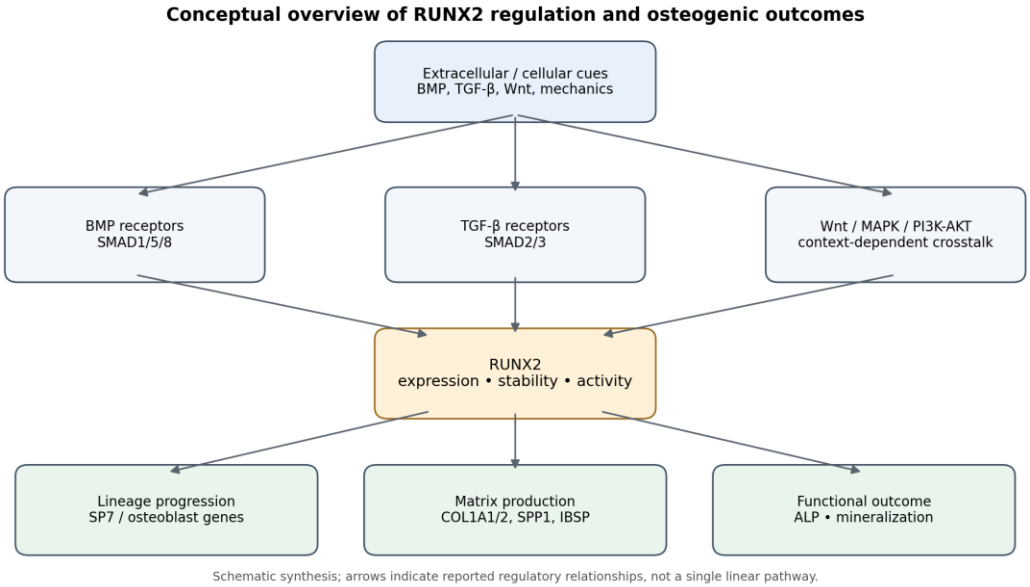


FIGURE 1: Conceptual overview of signaling pathways that regulate RUNX2 and downstream osteogenic outcomes.

3.1. BMP/SMAD signaling

Bone morphogenetic protein (BMP) signaling is an important regulator of osteogenic differentiation. Following BMP ligand binding to its receptors, receptor-regulated SMAD1/5/8 proteins become phosphorylated and participate in transcriptional regulation. Yun et al. [6] reported that suffruticosol A (Suf-A) increased RUNX2 expression through BMP2-associated SMAD1/5/8 signaling and MAPK activation in preosteoblasts. The involvement of BMP2 was supported by the use of Noggin, a BMP antagonist, which attenuated the osteogenic response.

Piao et al. [11] demonstrated that cyclophilin A (CypA) enhanced BMP4-induced osteogenic differentiation in C2C12 cells. After C2C12 cells reached confluence, differentiation was induced with BMP4 in DMEM containing 2% FBS for 72 hours. CypA overexpression increased ALP activity and ALP/BSP/OC promoter activity, whereas CypA knockdown reduced these responses. CypA interacted directly with RUNX2, enhanced RUNX2 transcriptional activity and DNA binding to OSE2, and was associated with Akt signaling. This study evaluated early differentiation and transcriptional activity; it did not report a long-term mineralization assay.

These findings indicate that BMP signaling can interact with RUNX2 regulation at more than one level. However, the direction and magnitude of the response depend on the experimental model and the molecular regulator being investigated [6,11].

3.2. Wnt/β-catenin signaling

Canonical Wnt signaling begins with ligand binding to Frizzled and LRP5/6 receptors, which promotes β-catenin stabilization and nuclear translocation. Nuclear β-catenin contributes to transcriptional programs involved in osteoblast-lineage progression. The relationship between Wnt signaling and RUNX2 is context-dependent and involves coordinated regulation rather than an identical response in every model [1,2].

In primary murine osteoblasts, Ahmad et al. [8] showed that mechanical stimulation increased Wnt1/β-catenin signaling and the expression of Runx2 and Sp7 through a pathway involving tissue-type plasminogen activator (Plat). Wnt1 or Plat knockdown attenuated these responses, providing experimental support for mechanically regulated canonical Wnt signaling in osteoblast differentiation. The authors also observed reduced Wnt1 and Plat expression in bones from ovariectomized and aged mouse models.

The findings of Ahmad et al. [8] support a mechanistic connection between mechanical stimulation, Wnt1/β-catenin activity, Plat, and osteogenic transcription factors. They should, however, be interpreted within the primary murine osteoblast and mechanical-loading model used in that study.

3.3. MAPK signaling

The mitogen-activated protein kinase (MAPK) family includes ERK, p38, and JNK pathways, which regulate cellular responses to extracellular signals and can influence osteogenic transcriptional activity. Yun et al. [6] reported increased MAPK phosphorylation alongside enhanced RUNX2 expression during preosteoblast differentiation following Suf-A treatment. Yadav et al. [10] found that orsellinic-acid-loaded gelatin/nanohydroxyapatite /chitosan nanoparticle scaffolds stimulated integrin α2 and β1 expression, followed by FAK and ERK activation and increased RUNX2-associated osteogenic differentiation.

Mechanical stimulation can also activate MAPK-associated responses. Binlateh et al. [9] reported that low-magnitude compressive force (1 and 2 g/cm^2 for 24 hours) promoted osteoblast differentiation and mineral deposition, accompanied by increased RAMP1 expression and p38 phosphorylation. Higher force magnitudes (3 and 4 g/cm^2) did not produce the same osteogenic effect. The study also showed that calcitonin gene-related peptide (CGRP), a RAMP1 ligand, promoted osteogenic responses.

Kim et al. [19] further demonstrated that MCP-3 promoted osteoblast differentiation through CCR3-dependent p38 phosphorylation. Primary osteoblast precursor cells isolated from neonatal mouse calvariae were cultured in medium containing BMP2 (100 ng/mL), ascorbic acid (50 μg/mL), and β-glycerophosphate (10 mM), with or without MCP-3 (100 ng/mL). MCP-3 increased ALP activity after three days and matrix mineralization after six days, and increased Alpl and Ibsp expression; however, it did not increase Runx2 mRNA. Accordingly, this study supports an osteogenic role for MCP-3 through CCR3/p38 signaling, but it does not support a claim that MCP-3 directly upregulates RUNX2.

3.4. PI3K/AKT signaling

PI3K/AKT signaling contributes to cell survival, growth, and differentiation. Piao et al. [11] demonstrated that CypA interacted with RUNX2 and enhanced RUNX2-related transcriptional activity in BMP4-treated C2C12 cells, with AKT signaling implicated in this process. Piao et al. [12] subsequently reported that cyclophilin E (CypE) positively regulated osteoblast differentiation by increasing RUNX2 transcriptional activity through its peptidyl-prolyl cis-trans isomerase activity. The AKT pathway played an important role in the CypE-mediated response. These studies suggest that cyclophilin family proteins may regulate RUNX2 function beyond altering its expression level. Their findings should be interpreted cautiously because C2C12 cells are myoblast-lineage cells induced toward an osteogenic phenotype by BMP4, rather than primary osteoblasts [11,12].

3.5. TGF-β signaling and pathway interactions

Transforming growth factor beta (TGF-β) and bone morphogenetic proteins (BMPs) belong to the TGF-β superfamily, but they activate distinct receptor complexes and receptor-regulated SMADs. TGF-β commonly signals through SMAD2/3, whereas canonical BMP signaling involves SMAD1/5/8.

Both pathways also engage non-canonical signaling, including MAPK and PI3K-related mechanisms [23]. Their effects on osteoblast-lineage cells are stage-dependent: TGF-β can support early progenitor proliferation and osteogenic commitment but inhibit aspects of later osteoblast maturation; BMP signaling also has context-dependent effects on progenitor expansion, differentiation, and matrix mineralization [23]. RUNX2 is one of the transcriptional nodes through which these pathways influence osteogenic gene expression. Nevertheless, the primary studies in the 15-study comparison do not directly establish a specific TGF-β-mediated mechanism in their experimental models. The pathway-level interpretation in this subsection is therefore based on the review by Wu et al. [23], rather than being presented as a finding of the included primary-study set.

4. Post-transcriptional and epigenetic regulation of RUNX2

RUNX2 expression and activity are regulated not only at the transcriptional level but also through non-coding RNAs, chromatin modification, and post-translational mechanisms. Non-coding RNAs can influence RUNX2 expression by affecting mRNA stability or translation [20]. Ding et al. [13] reported that miR-224-5p was downregulated during osteogenic differentiation of C2C12 cells. Overexpression of miR-224-5p inhibited ALP activity, matrix mineralization, and osteogenic marker expression. The study identified Runx2 and Sp7 as direct targets of miR-224-5p, while inhibition of miR-224-5p attenuated ovariectomy-associated bone loss in mice.

Wang et al. [14] described a circ_0054633/miR-590-3p/RUNX2 regulatory loop in human mandibular bone marrow-derived mesenchymal stem cells (BMSCs), including cells obtained from individuals with type 2 diabetes mellitus (T2DM). The study found lower circ_0054633 expression in T2DM-derived BMSCs. Knockdown of circ_0054633 reduced ALP staining and activity, Alizarin Red S staining, calcium deposition, and ALP/OCN expression, whereas overexpression produced the opposite effects. RUNX2 was identified as a direct target of miR-590-3p, and RUNX2 also activated the circ_0054633 promoter, forming a positive feedback loop. The study assessed osteogenic marker mRNA across days 0, 3, 7, 14, and 21, ALP activity at day 7, mineralization at day 14, and protein expression after 10 days of induction. The findings are specific to the human mandibular BMSC and T2DM-related model and should not be generalized to all MSC sources.

Epigenetic regulation also contributes to RUNX2 activity. Chen et al. [16] demonstrated that the histone acetyltransferase MOF (KAT8) and histone H4K16 acetylation increased during osteoblast differentiation. MOF knockdown or inhibition with MG149 reduced Runx2 and Osterix expression and transactivation, whereas MOF overexpression enhanced their protein levels. Chromatin immunoprecipitation and protein interaction experiments supported MOF binding to the Runx2 and Osterix promoter regions and physical interaction with both transcription factors.

Post-translational modification provides another regulatory layer. Wu and Gong [15] reported that scopolamine promoted RUNX2 lactylation at lysine 176 (K176) in human periodontal ligament stem cells, reduced RUNX2 ubiquitination, and increased RUNX2 protein stability. RUNX2 knockdown attenuated the osteogenic response, linking this modification to ALP activity and matrix mineralization. Dong et al. [4] similarly showed that FXR stabilized RUNX2 by suppressing Thoc6-mediated ubiquitination. Collectively, these findings indicate that RUNX2 regulation involves interactions among transcriptional control, non-coding RNAs, chromatin regulation, and protein stability [4,13–16,20].

5. RUNX2 target genes and downstream osteogenic markers

RUNX2 is associated with the expression of several genes involved in osteoblast-lineage progression and bone matrix formation. SP7 encodes Osterix, a transcription factor involved in the progression of osteoblast differentiation. COL1A1 and COL1A2 encode the chains of type I collagen, which form the principal organic framework of bone matrix. Qin et al. [3] demonstrated that deletion of RUNX2 reduced the expression of both collagen genes in committed osteoblasts.

BGLAP encodes osteocalcin (OCN), which is associated with osteoblast function and mineralized matrix. SPP1 encodes osteopontin (OPN), while IBSP encodes bone sialoprotein (BSP); both encode non-collagenous matrix proteins involved in matrix organization and mineralization. Alkaline phosphatase (ALP) contributes to the availability of inorganic phosphate and is widely used as an indicator of osteogenic activity. These markers reflect different biological processes and should not be treated as interchangeable measures of osteogenesis [3,6,10,13,17,18].

The term RUNX2-associated genes is used here to describe genes whose expression is influenced by RUNX2 or is linked to RUNX2-dependent osteogenic programs. The evidence that a gene is a direct transcriptional target differs among genes and experimental systems. For example, Ding et al. [13] identified Runx2 and Sp7 as direct targets of miR-224-5p, whereas Qin et al. [3] demonstrated reduced matrix gene expression after conditional Runx2 deletion. Expression changes in an individual downstream marker do not independently demonstrate complete osteoblast maturation or successful mineralization.

6. RUNX2 in osteoblast maturation and bone matrix mineralization

RUNX2 contributes to bone formation through the regulation of genes associated with extracellular matrix production and osteoblast function. Type I collagen provides the organic framework of bone matrix, whereas non-collagenous proteins such as

osteocalcin, osteopontin, and bone sialoprotein contribute to matrix organization and mineralization. Qin et al. [3] showed that RUNX2 deletion in committed osteoblasts reduced the expression of several matrix protein genes and impaired bone formation in mice.

RUNX2 expression must nevertheless be distinguished from the actual deposition of mineralized matrix. Mineralization is a functional outcome that requires independent evaluation through methods such as Alizarin Red S staining, Von Kossa staining, or calcium quantification. Several included studies combined RUNX2-related measurements with these functional outcomes [6,10,13,17]. The relationship between RUNX2 and osteoblast maturation therefore depends on temporal regulation and coordination with other osteogenic factors; molecular expression data should be interpreted alongside functional outcomes [3,5,7].

7. Experimental approaches for evaluating RUNX2

RUNX2 can be evaluated at the gene and protein levels, but each analytical method provides different information. Reverse-transcription polymerase chain reaction (RT-PCR) and quantitative real-time PCR (qPCR) measure RUNX2 mRNA expression and provide evidence of transcriptional changes. These methods do not directly establish protein abundance, subcellular localization, or transcriptional activity. Several studies used qPCR or RT-PCR to evaluate Runx2 and related osteogenic genes, including Qin et al. [3], Yun et al. [6], Ding et al. [13], and Ahmad et al. [8].

Western blotting measures RUNX2 protein abundance in a sample, whereas immunofluorescence and immunocytochemistry provide information about protein localization and distribution. Reporter assays can assess transcriptional activity, while chromatin immunoprecipitation and protein-interaction assays can investigate regulatory interactions. These approaches were used in studies examining CypA/CypE, MOF, and RUNX2 regulation [11,12,16]. ELISA can quantify RUNX2 protein concentration in a biological sample when a validated assay is available; however, ELISA does not independently demonstrate nuclear localization, DNA-binding activity, or transcriptional function.

Functional osteogenic outcomes require complementary assessments. ALP activity assays, downstream osteogenic marker measurements, and mineralization staining provide information that cannot be obtained from RUNX2 measurement alone. Studies such as Yadav et al. [10], Alghofaily et al. [17], and Bayram et al. [18] illustrate the value of combining gene-expression analysis with cellular and mineralization outcomes. Combining molecular and functional methods is therefore preferable when evaluating osteogenic differentiation.

8. Factors influencing RUNX2 expression and activity

RUNX2 regulation is influenced by extracellular signals, mechanical conditions, epigenetic mechanisms, non-coding RNAs, and post-translational protein modification. The studies discussed above show that these influences may act at different regulatory levels: BMP and MAPK signaling can affect osteogenic transcriptional responses [6,9,10]; mechanical stimulation can alter p38- or Wnt-associated signaling [8,9]; and MOF-mediated histone acetylation, microRNAs, circular RNAs, RUNX2 lactylation, and ubiquitination can affect gene expression or protein activity [4,13–16,20].

The response is not uniform across all conditions. Kim et al. [19], for example, found that MCP-3 promoted osteoblast differentiation through CCR3/p38 signaling without increasing Runx2 mRNA. Binlateh et al. [9] found that the osteogenic effect of compressive force depended on its magnitude. These findings indicate that RUNX2 expression and activity are not controlled by a single pathway. The magnitude and direction of a response depend on the experimental stimulus, cell source, differentiation stage, and molecular endpoint measured.

9. RUNX2 in bone tissue engineering and biomaterial-based osteogenesis

RUNX2 is relevant to bone tissue engineering because it provides a molecular readout of osteogenic commitment and differentiation. Biomaterial studies have investigated combinations of chitosan, gelatin, and hydroxyapatite as platforms to support cell attachment and osteogenic responses. However, findings obtained with one formulation cannot automatically be transferred to another composition, fabrication method, bioactive additive, or cross-linking condition.

Yadav et al. [10] investigated gelatin/nanohydroxyapatite scaffolds containing orsellinic-acid-loaded chitosan nanoparticles using C3H10T1/2 mouse mesenchymal stem cells. The scaffold was fabricated by lyophilization and cross-linked with EDC; orsellinic acid was tested at 40, 80, and 120 μM, with 80 μM identified as the effective condition. RUNX2 mRNA and protein were assessed at days 7 and 14, alongside ALP and other osteogenic markers; Alizarin Red S and von Kossa staining were used to assess mineralization. The osteogenic response to the OA-containing scaffold was associated with integrin-mediated FAK and ERK activation. Because the formulation contained orsellinic acid, chitosan nanoparticles, gelatin, and nanoHA, the response should not be attributed to a single scaffold component.

Alghofaily et al. [17] studied chitosan–gelatin (CH-G) scaffolds incorporating valproic acid (VPA) and evaluated immortalized human bone marrow mesenchymal stem cells (hTERT-MSCs). The scaffold was prepared from 2% chitosan and 3% gelatin and loaded with VPA at 1, 3, or 5 mM. More than 90% of the cumulative VPA release occurred within seven days. RUNX2, osteocalcin (OCN), osteonectin, collagen type I, and ALP gene expression were assessed by qRT-PCR on days 9 and 12; ALP activity was assessed on days 9 and 14, and

mineralized nodules were evaluated by Alizarin Red S on day 12. The 1 mM VPA group showed the most favorable osteogenic response, including increased RUNX2 and OCN expression at the reported day-12 assessment. Because VPA was incorporated into the scaffold, the response cannot be attributed to CH-G composition alone.

Bayram et al. [18] investigated microfluidically fabricated gelatin and gelatin–nano-hydroxyapatite (nanoHA) scaffolds using dental pulp stem cells (DPSCs). The overall scaffold porosity exceeded 60%. Cell viability was assessed on days 3, 7, and 14, and ALP activity on days 7 and 14. The authors measured RUNX2 mRNA on day 7, BMP2 mRNA on day 14, and OCN mRNA on day 21. RUNX2 expression was significantly higher in the gelatin-only group than in both the control and gelatin–nanoHA groups, whereas OCN expression was higher in both scaffold groups than in the control, with the gelatin–nanoHA group exceeding gelatin alone. These different marker patterns show that RUNX2, ALP, BMP2, and OCN responses depend on both scaffold composition and observation time.

These studies illustrate the value of evaluating RUNX2 in biomaterial-supported osteogenic differentiation. They also show why scaffold composition, bioactive additives, fabrication methods, and observation time must be considered when interpreting osteogenic outcomes. Conclusions about a particular biomaterial formulation should be drawn only when that formulation has been directly tested.

10. Challenges, limitations, and conflicting findings

A central interpretive issue is the stage-dependent role of RUNX2 and the continuing debate over its function in committed osteoblasts. Conditional-deletion studies have produced divergent results. Takarada et al. [26] reported no overt skeletal phenotype after deleting the Runt domain in osteoblasts using a 2.3-kb Col1a1-Cre line, whereas Adhami et al. [22], using the same Cre line to delete exon 8, reported progressive postnatal growth impairment, reduced bone mass, and diminished bone formation. Komori [21] notes that exon 8 deletion produces a truncated RUNX2 protein that retains DNA binding but has reduced transcriptional activation, and that this protein may have interfered with RUNX3 function. Qin et al. [3] subsequently deleted the Runt domain using a newly generated Col1a1-Cre line and reported reduced bone parameters and lower expression of major matrix-protein genes. The outcome therefore differs between two Runt-domain deletion models [3,26], and the reason remains unresolved.

Together, these findings support a continuing contribution of RUNX2 after lineage commitment, but the literature should not be presented as uniform.

A second challenge is the heterogeneity of experimental models and methods. The studies reviewed used primary osteoblasts, preosteoblast cell lines, myoblast-lineage cells induced toward osteogenesis, bone marrow-derived stem cells, periodontal ligament stem cells, and dental pulp stem cells. Culture conditions, sampling times, experimental stimuli, and analytical methods also varied. RUNX2 mRNA expression, protein abundance, localization, DNA binding, and transcriptional activity are distinct measurements, limiting direct comparisons between studies. Some studies also lacked long-term functional outcomes such as matrix mineralization.

A further limitation is that several studies investigated specific bioactive compounds or molecular interventions rather than scaffold composition alone. The osteogenic effects reported for orsellinic acid, valproic acid, suffruticosol A, scopolamine, and mechanical stimulation cannot be generalized directly to an unmodified scaffold [6,9,10,15,17]. In addition, C2C12 cells are myoblast-lineage cells induced toward an osteogenic phenotype, and their findings should not be treated as equivalent to results from primary osteoblasts [11–13].

11. Research gaps and future directions

Further research should characterize RUNX2 expression and activity across successive stages of osteoblast differentiation. Longitudinal studies should combine RUNX2 mRNA, protein abundance, and localization with downstream osteogenic markers and functional mineralization assays. Additional experiments are needed to clarify interactions among BMP/SMAD, TGF-β/SMAD2/3, MAPK/ERK, Wnt/β-catenin, and PI3K/AKT signaling using appropriately controlled pathway activation and inhibition.

For biomaterial research, standardized experimental approaches should distinguish the effects of scaffold composition, bioactive additives, fabrication methods, and cross-linking conditions. Future studies should report physicochemical properties, cytocompatibility, cell source, culture conditions, sampling times, RUNX2 assay type, downstream markers, and functional mineralization outcomes. Material-specific conclusions should be drawn only when the relevant formulation and cross-linking agent have been tested directly.

TABLE 1: Integrated Synthesis of Runx2 Regulation and Osteogenic Outcomes.

Regulatory level / pathway	Evidence represented in this review	Interpretive point
BMP/SMAD and TGF-β	BMP2/SMAD1/5/8-associated responses (Yun et al. [6]); broader TGF-β/BMP mechanisms summarized by Wu et al. [23].	BMP and TGF-β are related superfamily pathways but use distinct R-SMADs and show stage-dependent effects.
Wnt/β-catenin and mechanical signaling	Wnt1/β-catenin/Plat signaling under laminar flow (Ahmad et al. [8]); RAMP1/p38 responses to compression (Binlateh et al. [9]).	Mechanical context and stimulus magnitude influence osteogenic outcomes.
MAPK and PI3K/AKT	ERK/p38-associated responses and cyclophilin-mediated RUNX2 activity (Yun et al. [6], Binlateh et al. [9], Piao et al. [11,12]).	Pathway activation does not necessarily imply direct RUNX2 transcriptional upregulation.
RNA and epigenetic regulation	miR-224-5p, circ_0054633/miR-590-3p, MOF/H4K16 acetylation (Ding et al. [13], Wang et al. [14], Chen et al. [16]).	RUNX2 can be regulated through transcript stability, translation, and chromatin state.
Protein stability and modification	RUNX2 lactylation and reduced ubiquitination; FXR/Thoc6-mediated protein stabilization (Wu and Gong [15], Dong et al. [4]).	Protein abundance may change independently of RUNX2 mRNA.
Downstream / functional outcomes	ALP, SP7, COL1A1/2, BGLAP, SPP1, IBSP, and mineralization assays across primary studies [3,6,10,13,17–19].	Gene/protein measurements should be interpreted alongside functional matrix outcomes.

CONCLUSION

RUNX2 is a central transcriptional regulator of osteoblast-lineage commitment and bone formation. Experimental and clinical evidence supports its importance during early osteogenic differentiation and its continuing contribution to bone matrix gene expression and postnatal skeletal maintenance. RUNX2 is regulated by BMP/SMAD, TGF-β-associated, Wnt/β-catenin, MAPK, and PI3K/AKT-related signaling, as well as by non-coding RNAs, chromatin modification, and post-translational mechanisms. Human cleidocranial dysplasia provides clinically relevant evidence linking pathogenic RUNX2 variants with skeletal and dental abnormalities. In biomaterial-supported osteogenesis, RUNX2 is a useful molecular readout, but the scaffold studies reviewed show that responses depend on composition, bioactive additives, fabrication, and sampling time, and therefore require functional mineralization assays [10,17,18]. Importantly, RUNX2 expression alone cannot establish complete osteoblast maturation or successful matrix mineralization. Interpretation must account for differentiation stage, cell model, and the molecular endpoint measured, and should be supported by complementary osteogenic and functional assays.

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