Review

Rho GTPases in Bone and Tooth Development and Diseases

Xiaofang Wang *, Sanjaya Thapa, Bikash Lamichhane, Yongxu Zhang

Posted Date: 21 April 2026

doi: 10.20944/preprints202604.1426.v1

Keywords: GTPase; RhoA; Rac1; Cdc42; bone; osteogenesis; dentinogenesis; amelogenesis; tooth development; craniofacial suture; osteoclast; osteoblast; skeletal stem cell

 doi:10.20944/preprints202604.1426.v1

Review

Rho GTPases in Bone and Tooth Development and Diseases

Xiaofang Wang [1,*], Sanjaya Thapa [1], Lamichhane Bikash [2] and Yongxu Zhang [1]

[1] Department of Biomedical Sciences, College of Dentistry, Texas A&M University, Dallas, TX, USA 75246

[2] Craniofacial Anomalies & Regeneration Section, National Institute of Dental & Craniofacial Research, Bethesda, MD, USA 20892

* Correspondence: xfwang@tamu.edu; Tel.: +1-214-370-7229; Fax: +1-214-874-4538

Abstract

Rho GTPases—including RhoA, Rac1, and Cdc42—are key molecular switches that regulate cytoskeletal dynamics and transduce biochemical and mechanical signals essential for skeletal and dental tissue development. These small GTPases orchestrate fundamental cellular processes such as proliferation, migration, polarity, and differentiation, thereby guiding the morphogenesis, homeostasis, and regeneration of bone and teeth. In bone, Rho GTPases modulate osteoblast proliferation and matrix mineralization, osteoclast-mediated bone resorption, and mechanotransductive responses to physical stimuli. They are also critical for the behavior and fate specification of skeletal stem cells, integrating environmental cues to balance self-renewal and lineage commitment. In dental tissues, Rho GTPases regulate epithelial–mesenchymal interactions, odontoblast and ameloblast polarization, and the formation of enamel and dentin. Additionally, they play vital roles in craniofacial suture development, where their spatially and temporally controlled activity maintains suture patency and regulates ossification. Dysregulation of Rho GTPase signaling is implicated in a variety of pathological conditions, including osteoporosis, craniosynostosis, and dentinogenesis and amelogenesis imperfecta. Despite their therapeutic potential, targeting Rho GTPases remains challenging due to their pleiotropic functions and broad tissue distribution. This review highlights the mechanistic roles, regulatory networks, and developmental relevance of RhoA, Rac1, and Cdc42 in skeletal and dental biology, and discusses emerging strategies for modulating their activity in regenerative and disease contexts.

Keywords: GTPase; RhoA; Rac1; Cdc42; bone; osteogenesis; dentinogenesis; amelogenesis; tooth development; craniofacial suture; osteoclast; osteoblast; skeletal stem cell

1. Introduction

The formation of bones and teeth is governed by a highly coordinated series of events that involve intricate genetic programming, signaling cascades, and mechanical stimuli. These developmental processes depend on dynamic interactions between mesenchymal progenitors, epithelial cells, and the extracellular matrix (ECM), ensuring the proper morphogenesis and mineralization of skeletal and dental tissues. In the skeletal system, mesenchymal stem cells differentiate into osteoblasts responsible for bone matrix production, while osteoclasts maintain bone homeostasis through resorption [1,2]. Tooth development is driven by reciprocal epithelial–mesenchymal interactions that direct the formation of ameloblasts and odontoblasts, the key cell types responsible for enamel and dentin production, respectively [3,4].

A central regulatory node in these processes is the family of Rho GTPases—particularly RhoA, Rac1, and Cdc42—which act as intracellular molecular switches linking extracellular signals to cytoskeletal reorganization, vesicle trafficking, and gene expression [5]. These small GTPases are tightly regulated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs), allowing for precise control over cell behavior [6]. Through their downstream effectors, Rho

 doi:10.20944/preprints202604.1426.v1

GTPases orchestrate actin dynamics, cell polarity, migration, and differentiation—functions essential for shaping the architecture of developing bones and teeth [7].

Emerging evidence underscores the vital roles of Rho GTPases in the activity of osteoblasts, osteoclasts, odontoblasts, and ameloblasts. Each member of this family plays distinct but overlapping roles: RhoA modulates mechanical signaling and contractility [8]; Rac1 facilitates membrane protrusion and directed movement [9]; and Cdc42 governs cellular polarity and organization [10]. Their influence extends to interactions with major signaling pathways such as Wnt/β-catenin, TGF-β/BMP, and YAP/TAZ, highlighting their integrative role in mediating both biochemical and biomechanical cues [11–14]. Dysregulation of Rho GTPase signaling contributes to various pathological conditions, including osteoporosis, osteoarthritis, and inherited defects of enamel and dentin [15–18].

This review synthesizes current insights into the molecular functions of RhoA, Rac1, and Cdc42 during bone and tooth development. It explores their upstream regulators, downstream signaling mechanisms, and mechanotransductive roles, emphasizing how their coordinated activity is crucial for normal tissue formation and homeostasis. We also examine the therapeutic potential of modulating Rho GTPase signaling for regenerative applications and the treatment of craniofacial and skeletal disorders.

2. Overview of Rho GTPases and Their Signaling Networks

Small GTPases are molecular switches that play fundamental roles in regulating a wide array of cellular processes including proliferation, migration, cytoskeletal dynamics, and polarity [19]. These proteins belong to the Ras superfamily, which is divided into five major subfamilies: Ras, Rho, Rab, Arf, and Ran [19]. Each subfamily controls distinct cellular functions, with the Rho family being particularly critical for cytoskeletal organization, cell adhesion, and morphogenetic behavior. Among the more than 20 known Rho family members, RhoA, Rac1, and Cdc42 are the best characterized and serve as central regulators of cell structure and behavior in developmental systems such as bone and teeth.

Like other members of the small GTPase family, Rho GTPases function by cycling between an inactive GDP-bound state and an active GTP-bound state. This transition is tightly controlled by upstream regulatory proteins: guanine nucleotide exchange factors (GEFs) catalyze the exchange of GDP for GTP to activate the GTPase; GTPase-activating proteins (GAPs) enhance the intrinsic GTPase activity to inactivate them; and guanine nucleotide dissociation inhibitors (GDIs) maintain the GTPases in an inactive state by preventing GDP dissociation and membrane localization [20]. Upon activation, Rho GTPases bind to specific downstream effectors that mediate various signaling events, resulting in context-dependent and cell type-specific biological outcomes. The diversity of regulatory proteins and effectors contributes to the complexity of Rho GTPase signaling networks, which are still being unraveled in vivo.

RhoA promotes actomyosin contractility and facilitates the formation of stress fibers and focal adhesions [21]. It acts primarily through downstream effectors such as Rho-associated kinase (ROCK) and mDia1, which modulate cytoskeletal tension, cell rounding, adhesion, and gene expression [22]. ROCK-mediated phosphorylation of myosin light chain (MLC) enhances actomyosin activity and is essential for generating contractile force during cell movement and tissue remodeling [23]. Additionally, RhoA influences transcription via the activation of serum response factor (SRF), linking cytoskeletal dynamics to gene regulatory programs [24].

Rac1 controls the formation of lamellipodia—broad, sheet-like membrane protrusions rich in branched actin networks—by activating the WAVE complex and subsequently the Arp2/3 complex, which nucleate actin branching [25]. Rac1 is essential for membrane ruffling, cell spreading, and directional migration [26]. Beyond its structural functions, Rac1 regulates intracellular signaling pathways including NF-κB and JNK, influences epithelial integrity by modulating cell-cell junctions, and contributes to the generation of reactive oxygen species (ROS), especially in immune and

 doi:10.20944/preprints202604.1426.v1

neuronal cells [27–29]. In skeletal and dental systems, Rac1's control over actin reorganization and gene transcription is crucial for proper differentiation and morphogenesis.

Cdc42 plays a pivotal role in establishing cell polarity and guiding directional movement. It induces the formation of filopodia—slender, actin-rich protrusions that serve sensory and exploratory functions—by activating WASP family proteins, which also stimulate Arp2/3-dependent actin polymerization [30]. Cdc42 also interacts with the Par6–aPKC complex to orient the Golgi apparatus and the microtubule-organizing center, enabling spatially directed trafficking and asymmetric cell division [31]. In addition, Cdc42 is involved in regulating vesicular trafficking, mitotic spindle orientation, and cell cycle progression, all of which contribute to tissue patterning and organ development [31–33].

Although RhoA, Rac1, and Cdc42 each have distinct primary functions, they frequently operate in a coordinated and sequential manner. For example, during directional cell migration, Cdc42 is typically activated at the leading edge to establish front–rear polarity [34], Rac1 drives forward protrusion via lamellipodia [35], and RhoA provides contractile force at the cell rear to stabilize adhesion and maintain directional movement [36]. This spatial and temporal coordination enables cells to interpret and respond to extracellular cues with remarkable precision.

3. Rho GTPases in Bone Biology

3.1. Rho GTPases in Osteogenesis

RhoA, Rac1, and Cdc42 serve as key molecular integrators that regulate cytoskeletal architecture, cellular polarity, mechanotransduction, and gene expression during osteogenesis. Acting as mechanosensitive signaling hubs, they coordinate ECM cues and intracellular pathways to govern the fate and function of skeletal stem cells (SSCs) and osteoblasts [37–39].

RhoA functions as a finely tuned rheostat that interprets ECM stiffness and geometry through the integrin–FAK axis, activating downstream effectors such as ROCK and mDia [40,41]. This cascade enhances actomyosin contractility, focal adhesion assembly, and nuclear translocation of YAP/TAZ and MRTF-A—transcriptional coactivators that drive the expression of osteogenic genes like *Runx2* and *Sp7* [42,43]. While RhoA promotes osteogenesis under physiological conditions, its hyperactivation—especially in aging or pathological contexts—can suppress Wnt/β-catenin signaling and inhibit differentiation [44,45], highlighting the need for balanced activity.

Rac1 plays a pivotal role in skeletal morphogenesis, osteoblast function, and SSC behavior. It regulates lamellipodia formation, cell migration, and focal adhesion turnover, and enhances osteoblast responsiveness to mechanical and biochemical cues via the PAK–MAPK and Wnt/β-catenin pathways [46,47]. In SSCs, Rac1 controls migration, polarity, and lineage commitment, promoting osteogenesis while suppressing alternative fates [38,48]. It also mediates responses to growth factors and ECM composition, making it a critical modulator of bone homeostasis and regeneration [38,47].

Cdc42 similarly coordinates cytoskeletal remodeling, Golgi polarization, and directed vesicular trafficking during osteoblast differentiation [32,33]. It activates PAK–LIMK1 and ERK/Smad pathways to regulate *Runx2* expression and osteogenic function [49,50]. In SSCs, Cdc42 governs quiescence, fate decisions, and mechanotransduction, and its dysregulation contributes to skeletal aging and joint degeneration [51,52]. Its upstream activators, such as the GEF FGD1, further underscore its clinical relevance, as mutations in *FGD1* lead to skeletal anomalies seen in Aarskog syndrome [53,54].

Together, these Rho GTPases act as dynamic orchestrators of bone development and remodeling, translating physical and chemical signals into precise cellular outcomes. Their complex, context-dependent roles offer promising yet nuanced targets for therapeutic strategies aimed at enhancing bone regeneration and treating skeletal disorders.

 doi:10.20944/preprints202604.1426.v1

3.1.1. RhoA in Osteogenesis

RhoA plays a central and multifaceted role in the regulation of osteogenesis and SSC fate. Far from acting as a simple molecular switch, RhoA functions as a finely tuned rheostat that integrates extracellular mechanical signals with intracellular biochemical networks to guide bone formation across multiple stages—from stem cell lineage commitment to osteoblast maturation and matrix mineralization [7,55–57]. Through downstream effectors such as ROCK and mDia, RhoA governs actomyosin contractility, cytoskeletal architecture, and focal adhesion dynamics [55,58], while also facilitating the nuclear translocation of mechanosensitive transcriptional regulators such as YAP/TAZ and MRTF-A [59–61]. These transcriptional coactivators, once in the nucleus, drive the expression of key osteogenic genes including *Runx2*, *Sp7 (Osterix)*, *BGLAP* (osteocalcin), and *SPP1* (osteopontin), thereby directing SSCs toward the osteoblast lineage and supporting the functional maturation of osteoblasts [61–63].

One of the most upstream and influential regulators of RhoA in SSCs and osteoblasts is the extracellular matrix (ECM). ECM components such as fibronectin, collagen, and vitronectin engage integrins—transmembrane adhesion receptors that physically connect the matrix to the cytoskeleton through focal adhesions [64]. Upon ligand binding and clustering, integrins activate focal adhesion kinase (FAK), which recruits Src family kinases and guanine nucleotide exchange factors (GEFs) that convert inactive GDP-bound RhoA to its active GTP-bound form [55,64]. This ECM–integrin–FAK–RhoA axis serves as a critical signaling cascade that allows cells to translate extracellular stiffness and topographical cues into intracellular tension and gene expression. On stiff substrates or mechanically active environments, enhanced RhoA activation leads to actin stress fiber formation, increased focal adhesion assembly, and MRTF-A and YAP/TAZ translocation into the nucleus, collectively driving osteogenic differentiation [40,41,61].

In SSCs, RhoA acts not only as a permissive factor but also as an instructive signal that determines lineage fate based on mechanical and geometrical input. When SSCs adhere to stiff matrices or occupy elongated morphologies, RhoA becomes activated, increasing cytoskeletal tension and promoting the osteogenic transcriptional program[57,65,66]. In contrast, cells on soft matrices or with reduced spreading exhibit low RhoA activity and default toward adipogenic differentiation via upregulation of PPARγ and downregulation of osteogenic factors [65,66]. RhoA-ROCK-mediated phosphorylation of myosin light chain (MLC) further stabilizes the cytoskeleton and promotes chromatin remodeling, facilitating access to osteogenic enhancers [58,59]. Importantly, RhoA also interfaces with BMP2 signaling by reinforcing Smad1/5 activity and coordinating cytoskeletal tension with morphogen-induced transcription [67].

Beyond matrix stiffness, ECM geometry also modulates RhoA activation. In structured microenvironments such as curved surfaces, micropatterns, or 3D printed scaffolds, localized RhoA signaling enhances osteoblast alignment, matrix organization, and directional migration. Such spatial control of RhoA allows cells to interpret architectural features and translate them into patterned bone matrix deposition during regeneration [68,69].

Despite these largely anabolic effects, RhoA's role is not unidirectionally promotive. When RhoA activity is excessively high or chronically sustained—particularly in the absence of proper mechanical cues—it can become inhibitory. In conditions such as exposure to Pasteurella multocida toxin (PMT), osteoblast differentiation and mineralization are significantly suppressed through RhoA hyperactivation [70]. Interestingly, this inhibition can be reversed by C3 transferase (a RhoA inhibitor) or by pharmacologic ROCK blockade, and in some instances, ROCK inhibitors alone can enhance osteogenic differentiation, suggesting that there exists an optimal RhoA activation range beyond which osteogenesis is impaired [70].

In aging tissues, this regulatory balance becomes especially critical. Sustained RhoA activity in aged SSCs or stromal cells contributes to impaired osteogenesis through suppression of Wnt/β-catenin signaling. Mechanistically, elevated cytoskeletal tension enhances GSK-3β activity, leading to β-catenin degradation and limiting nuclear transcriptional activity essential for osteoprogenitor maintenance [44]. Similarly, in IFT80-deficient osteoprogenitors, RhoA hyperactivation through

 doi:10.20944/preprints202604.1426.v1

noncanonical Hedgehog signaling impairs differentiation via the cofilin/MLC2 pathway; partial inhibition of RhoA or ROCK restores osteogenic potential [45].

These inhibitory effects are not limited to pathological or aging contexts. Several physiological signaling pathways that enhance bone formation do so, at least in part, by actively downregulating RhoA. For example, EphB4-mediated forward signaling suppresses RhoA activity in osteoblasts, resulting in enhanced bone formation [63]. Likewise, Semaphorin 4D antagonism, Phactr1 downregulation, and treatment with annatto-derived tocotrienol each promote osteogenesis by attenuating RhoA or ROCK signaling, thereby enhancing BMP2 responsiveness and osteogenic gene expression [71–73].

These observations collectively position RhoA as a tightly regulated mechanosensitive integrator, rather than a simple on/off switch for osteogenesis. It is the amplitude, duration, spatial localization, and context of RhoA signaling that determine its biological outcome—whether promoting differentiation and regeneration or inducing repression and dysfunction [55–58]. This duality has important therapeutic implications: strategies aimed at modulating RhoA activity for bone regeneration must avoid indiscriminate activation or inhibition. Instead, targeted, context-aware modulation—through biomaterials that mimic physiological stiffness, small molecules that fine-tune cytoskeletal tension, or delivery systems responsive to mechanical environments—offers the most promise for harnessing RhoA to enhance skeletal repair, reverse age-related decline, and optimize stem cell-based therapies [40,61,74].

3.1.2. Rac1 in Osteogenesis

Rac1 is a pivotal regulator of skeletal development, osteoblast function, and SSC fate. Acting as a molecular switch, Rac1 orchestrates cytoskeletal dynamics, cell polarity, integrin-mediated adhesion, and mechanotransductive signaling. These functions are indispensable for early skeletal morphogenesis, bone matrix formation, and the precise coordination of SSC behavior in both homeostatic and regenerative contexts.

In embryonic development, Rac1 is essential for proper limb and craniofacial morphogenesis. Conditional knockout models reveal that deletion of Rac1 in mesenchymal or cartilage tissues results in growth plate disorganization, digit malformations, syndactyly, and severely shortened skeletal elements, underscoring its role in chondrogenesis, programmed cell death, and skeletal patterning. Rac1 influences ERK and caspase-3 signaling to control interdigital apoptosis and governs mesenchymal cell proliferation, polarity, and matrix organization necessary for endochondral ossification [75–79].

In osteoblasts, Rac1 promotes the formation of lamellipodia and filopodia, facilitating cell spreading, migration, and mechanosensing. These cytoskeletal features enable osteoblasts to interpret extracellular cues, respond to substrate stiffness, and engage in effective bone matrix deposition. Integrin-mediated Rac1 activation, particularly through fibronectin or CTGF interactions, supports focal adhesion assembly and activates downstream pathways including PAK and MAPK/ERK, which drive the expression of osteogenic genes such as Runx2, alkaline phosphatase (ALP), and osteocalcin [80–83]. Rac1 also enhances ERK-dependent phosphorylation of Runx2, reinforcing transcriptional programs central to osteogenesis [84,85].

Rac1's integration with Wnt/β-catenin signaling further amplifies its influence on osteogenesis. It stabilizes β-catenin, facilitates its nuclear translocation, and enhances its transcriptional activity in conjunction with TCF/LEF complexes. This crosstalk is crucial for osteoblast commitment and bone formation [86–88]. Notably, Rac1 augments canonical Wnt signaling not only by regulating β-catenin but also by influencing the actin cytoskeleton and nuclear complex assembly [88,89].

Beyond its role in differentiated osteoblasts, Rac1 is a central modulator of SSC biology. It regulates actin polymerization necessary for lamellipodia formation, enabling SSC migration through the bone marrow niche and toward regenerative sites. In vitro and in vivo, Rac1 activation via TAFA2-p38 signaling increases SSC motility, while Rac1-induced surface stiffness enhances 3D matrix penetration—both critical for tissue regeneration and injury repair [90,91]. Moreover, Rac1 is

 doi:10.20944/preprints202604.1426.v1

a key determinant of cell polarity and asymmetric division, essential for SSC self-renewal and spatially controlled lineage differentiation [92–94].

Rac1 supports SSC proliferation and survival by activating mitogenic and cytoskeletal programs through effectors like PAK and through growth factor pathways such as PDGF, IGF-1, and FGF2. These inputs converge on Rac1 to drive SSC expansion and metabolic resilience, especially under regenerative or aging-related stress [95–100]. Loss or inhibition of Rac1 in SSCs diminishes these signaling outputs, impairing proliferative potential and compromising regenerative outcomes [80,81,100].

Crucially, Rac1 controls SSC osteogenic commitment by enhancing BMP2 responsiveness and facilitating Runx2 and β-catenin activation. This regulation steers SSCs toward the osteoblast lineage while suppressing adipogenic and chondrogenic programs. Rac1-deficient SSCs show reduced osteogenic gene expression and increased alternative lineage differentiation, emphasizing its role as a molecular gatekeeper of osteoblast fate [86,88,101,102].

Interactions with the extracellular matrix further modulate Rac1 activity in SSCs. Rac1 links integrin engagement to actin remodeling, focal adhesion turnover, and mechanotransduction. ECM stiffness, geometry, and composition activate Rac1, allowing SSCs to sense and adapt to their physical environment. This enables SSCs to align with mechanical cues during development, load-induced bone remodeling, or tissue engineering [87,103–105]. Through integrin β1 and downstream PAK signaling, Rac1 also interfaces with YAP/TAZ activity, translating biomechanical signals into osteogenic transcription [38,106].

Rac1 activity must be precisely regulated. Hyperactivation, as observed with ARHGAP25 mutations, leads to early-onset skeletal fragility, while its aberrant signaling contributes to pathologic ossification in diseases such as ankylosing spondylitis via the CXCL12/CXCR4-Rac1 axis [48,102]. Conversely, moderate Rac1 inhibition has been shown to enhance BMP2-induced osteoblast differentiation and prevent pathological calcification, indicating a context-dependent duality in Rac1's function [101,107]. In osteoporosis models, Rac1 is a downstream mediator of spermine's protective effects on bone mass, suggesting therapeutic avenues via modulation of the Rac1–p53 axis [47].

Altogether, Rac1 serves as a master integrator of cytoskeletal remodeling, niche signaling, and transcriptional control in both SSCs and osteoblasts. Its dynamic regulation of migration, proliferation, mechanosensation, and lineage commitment underscores its indispensable role in skeletal development, bone homeostasis, and regenerative capacity. Strategically targeting Rac1 or its network may unlock new therapies for enhancing bone repair and treating disorders of skeletal fragility.

3.1.3. Cdc42 in Osteogenesis

Cdc42 functions as a pivotal regulator of cytoskeletal dynamics, cell polarity, and intracellular signaling, exerting essential roles in both osteogenesis and SSC biology. Its regulatory influence spans key developmental and homeostatic processes, including mesenchymal condensation, lineage commitment, osteoblast differentiation, and SSC maintenance under both physiological and stress-induced conditions [39,51,108,109].

During osteogenesis, Cdc42 orchestrates cytoskeletal remodeling by activating WASP-family proteins and promoting Arp2/3-dependent actin polymerization, thereby facilitating the formation of filopodia and other protrusive structures necessary for osteoblast migration and extracellular matrix (ECM) organization [32,34,110,111]. Concurrently, it regulates microtubule-dependent Golgi positioning and vesicular trafficking, enabling the polarized secretion of osteogenic factors [33,110]. Disruption of these processes impairs osteoblast alignment and matrix deposition, culminating in defective bone architecture and mineralization [39].

Cdc42 exerts its downstream effects via multiple signaling axes, including the PAK1–LIMK1 cascade [50,112], MAPKs (JNK and p38) [49,50], and the ERK/Smad pathway [49,52]. These converge on transcriptional effectors such as Runx2, a master regulator of osteoblastogenesis, thereby coupling

 doi:10.20944/preprints202604.1426.v1

cytoskeletal dynamics to osteogenic gene expression programs [49,113]. Upstream, the Cdc42-specific GEF FGD1 plays a critical role in this context; mutations in *FGD1* result in Aarskog syndrome, a developmental disorder characterized by skeletal anomalies, underscoring the clinical relevance of Cdc42-dependent signaling [53,54].

Cdc42 also integrates mechanical signals into osteogenic responses. In osteoblasts, fluid shear stress activates β-catenin signaling via Cdc42 and Rac1, promoting mechanoadaptive bone remodeling [114]. In SSCs, morphogenic cues such as BMP-2 and Activin B trigger Cdc42–PAK1–LIMK1 signaling and mDia1-mediated cytoskeletal rearrangements to enhance migratory behavior and osteogenic differentiation [110,112], highlighting its role in biomechanical and biochemical signal transduction.

Within the SSC compartment, Cdc42 is indispensable for preserving cytoskeletal polarity, metabolic fitness, and stem cell quiescence. Under stress conditions such as hematopoietic stem cell transplantation, aberrant elevation of Cdc42 activity disrupts actin organization and impairs mitophagy, leading to compromised niche function. Targeted pharmacological inhibition of Cdc42 reverses these defects, restoring SSC homeostasis and enhancing trabecular bone regeneration [51,109]. Moreover, lineage-tracing studies demonstrate that Cdc42 modulates fate determination: its deletion in osteoprogenitors biases differentiation toward the myeloid lineage, while its hyperactivation exacerbates osteoclastogenesis and bone loss [39,115]. These dual roles are further supported by evidence that Cdc42 mediates BMP-2-induced SSC migration and osteogenic commitment in vitro and in vivo [112].

Cdc42 also plays indispensable roles in skeletal development. Conditional ablation of Cdc42 in embryonic models results in defective mesenchymal condensation, syndactyly, disrupted chondrogenesis, and craniofacial abnormalities, reflecting its essential role in organizing chondrocyte polarity and actin dynamics [77,116]. Moreover, Cdc42 operates upstream of the CEP1–septin–actin axis, coordinating neural crest cell migration and morphogenetic patterning during early skeletal formation [117,118].

Pathologically, Cdc42 dysregulation contributes to aging-associated skeletal disorders and degenerative joint disease. In osteoarthritis (OA) models, increased Cdc42 activity in subchondral bone activates the JAK/STAT3 pathway, exacerbating cartilage matrix degradation and disrupting osteoblast–osteoclast coupling through Erk/Smad signaling [52,119]. Inhibition of Cdc42 in this context mitigates chondrocyte hypertrophy and restores subchondral bone homeostasis, identifying it as a promising therapeutic target for OA [52,119].

In sum, Cdc42 operates as a context-sensitive signaling hub that integrates biochemical, mechanical, and spatial cues to regulate SSC fate, osteogenic differentiation, and skeletal tissue integrity. Its multifaceted roles in bone development, remodeling, and regeneration position Cdc42 as a strategic target for therapeutic intervention in osteoporosis, skeletal aging, and osteoarticular disease [39,51,108,109].

3.2. Rho GTPases in Osteoclastogenesis and Bone Resorption

RhoA, Rac1, and Cdc42 are central regulators of osteoclastogenesis and bone resorption, functioning as molecular switches that coordinate cytoskeletal organization, vesicle trafficking, and signaling integration. Their tightly controlled activity governs each stage of osteoclast development—from precursor fusion and migration to polarization and matrix degradation—ensuring proper skeletal remodeling [120,121].

Rac1 and Cdc42 are predominantly active during early osteoclastogenesis. They promote actin polymerization and the organization of podosomes and lamellipodia, structures essential for osteoclast motility, fusion, and adhesion [122,123]. Rac1 enhances RANKL-induced signaling by promoting receptor clustering and activating NF-κB, MAPK, and JNK pathways, leading to upregulation of transcription factors such as NFATc1 and c-Fos [124,125]. Cdc42 supports similar roles by regulating MITF and NFATc1 expression and contributing to cytoskeletal polarization via the Par3/Par6/aPKC complex [115,126].

 doi:10.20944/preprints202604.1426.v1

In mature osteoclasts, RhoA becomes dominant. It maintains the sealing zone and ruffled border by activating ROCK, which promotes actomyosin contractility and podosome patterning [127,128]. RhoA also transduces matrix-derived signals via FAK/Src and modulates osteoclast response to mechanical cues through ROCK–YAP pathways [129]. Its spatial and temporal control by GEF-H1 and the mDia2–HDAC6 axis is critical for cytoskeletal stability [130,131].

Dysregulation of these GTPases impairs osteoclast function and causes skeletal disease. Loss of Rac1 or Cdc42 leads to osteopetrosis [115,132], while their hyperactivation accelerates bone loss in inflammatory settings [126,133].

Together, RhoA, Rac1, and Cdc42 form an interdependent regulatory triad essential for osteoclast-mediated resorption and bone homeostasis.

3.2.1. RhoA in Osteoclastogenesis and Bone Resorption

RhoA plays a central and multifaceted role in osteoclastogenesis and bone resorption by coordinating cytoskeletal organization, transcriptional control, mechanotransduction, and inflammatory signaling. Its function spans the entire osteoclast lifecycle—from early precursor commitment to the execution of bone-degrading activity—making it a critical molecular hub in both physiological bone remodeling and pathological bone loss [121,134].

In mature osteoclasts, RhoA is indispensable for the formation and stability of the actin-rich sealing zone and the ruffled border, which together define the resorptive microenvironment. Through activation of its downstream effector ROCK, RhoA phosphorylates myosin light chain (MLC), enhancing actomyosin contractility and generating the mechanical tension necessary for cytoskeletal stability and spatial compartmentalization [127,135]. This contractile force organizes podosomes—actin-rich adhesion structures that serve as sealing zone precursors—into dense rings that anchor osteoclasts to the bone surface [128,136].

Effective osteoclast polarization and cytoskeletal organization require precise spatial and temporal regulation of RhoA activity. Constitutive activation of RhoA fails to induce functional sealing zones and instead disrupts podosome organization, highlighting the detrimental effects of uncontrolled signaling [128,131,137]. Conversely, inhibition of RhoA with C3 exoenzyme eliminates sealing zones and causes podosomes to reorganize into peripheral belts, further underscoring RhoA's essential role in SZ assembly [127,137,138]. Spatial control of RhoA is mediated in part by microtubule-associated GEFs such as GEF-H1, which restrict RhoA activation to the bone interface where SZs form [130]. Temporally, RhoA signaling is modulated through effector pathways like the mDia2–HDAC6 axis, which links RhoA to microtubule acetylation and stabilizes the sealing zone architecture [131].

Beyond structural regulation, RhoA integrates critical signaling pathways governing osteoclast differentiation. Following RANKL-RANK interaction, RhoA facilitates receptor clustering and cytoskeletal rearrangement, enhancing activation of NF-κB and NFATc1. ROCK-mediated phosphorylation of IKKβ promotes IκBα degradation and nuclear translocation of NF-κB subunits, which in turn upregulate osteoclastogenic genes including NFATc1, TRAP, and cathepsin K [129,139–142]. Disruption of RhoA or ROCK function blunts this transcriptional response and impairs osteoclast differentiation and function [143,144].

RhoA also enables osteoclasts to respond dynamically to mechanical and matrix-derived signals. Engagement of αvβ3 integrins with bone matrix proteins activates FAK and Src, which then stimulate RhoA to initiate focal adhesion assembly and actin reorganization [145–147]. While RhoA activation generally promotes SZ formation, excessive substrate stiffness suppresses integrin β3–RhoA–ROCK–YAP signaling and paradoxically enhances osteoclastogenesis, revealing the context-dependent nature of RhoA mechanotransduction [129]. RhoA is also activated by extracellular cues such as GPR55 signaling through Gα13 in response to cannabinoid agonists, further promoting osteoclast polarization and resorption [148,149].

In non-canonical Wnt signaling, Wnt5a engages Ror2 to activate a RhoA-centered axis involving Daam2, Pkn3, and c-Src, driving actin ring formation and vesicular trafficking to enhance bone matrix

 doi:10.20944/preprints202604.1426.v1

degradation independently of β-catenin [150–152]. Conversely, regulatory pathways such as ERK5-mediated induction of RhoGAP7 serve to limit RhoA activation and shift the balance toward podosome assembly [128,153], highlighting the need for precise temporal downregulation in specific contexts. RhoA's influence on phosphoinositide metabolism—via $PI(4,5)P_2$ generation and WASp activation—further supports SZ formation [136].

Aberrant RhoA signaling contributes to diverse skeletal pathologies. In rheumatoid arthritis, RhoA amplifies NF-κB-driven inflammatory signaling and promotes joint erosion [29,42,154,155]. In osteoarthritis, RhoA activation through the Wnt/PCP pathway drives subchondral bone damage and cartilage degeneration [16,154,156,157]. Conversely, impaired RhoA activity due to disrupted microtubule interactions or GEF loss impairs SZ formation and osteoclast polarity, producing osteopetrosis-like features [127,130,158,159].

Given its central role in coordinating osteoclast function and its context-dependent duality, RhoA has emerged as a highly promising therapeutic target. Inhibitors of ROCK, such as Y-27632 and fasudil, reduce osteoclast formation and bone erosion in arthritis models [16,143]. Post-transcriptional approaches, including miR-31 and siRNA, downregulate RhoA expression to attenuate bone resorption [160,161]. Precision strategies like macrophage-targeted SMART-Cas9 enable selective gene editing of RhoA in inflamed joints, reducing osteoclastogenesis without disrupting physiological remodeling [155]. Furthermore, modulation of upstream cues—such as hyaluronic acid–CD44 engagement or Semaphorin 3A signaling—offers dual osteoclast-inhibitory and osteoblast-stimulatory effects [71,162,163].

Altogether, RhoA functions as a context-sensitive signaling rheostat that integrates matrix adhesion, mechanical cues, and cytoskeletal patterning to guide osteoclast differentiation, polarization, and resorption. Its tightly regulated spatial and temporal activity is essential for sealing zone formation and functional bone degradation. Translational approaches that preserve this regulatory precision while selectively targeting pathological overactivation represent a compelling frontier for treating bone loss disorders.

3.2.2. Rac1 in Osteoclastogenesis and Bone Resorption

Rac1 is a pivotal regulator of osteoclast biology, coordinating cytoskeletal dynamics, signal transduction, and gene expression to drive osteoclast differentiation, activation, and bone resorption. Its function is particularly critical in organizing the actin cytoskeleton, which underlies the complex structural transformations that osteoclasts undergo during bone matrix degradation. By regulating podosome formation, lamellipodia extension, and ruffled border development, Rac1 enables osteoclasts to migrate, adhere to bone surfaces, form sealing zones, and establish the acidic, proteolytic microenvironment necessary for bone resorption [122,132,134,164].

During osteoclastogenesis, Rac1 integrates signals downstream of RANKL and M-CSF to modulate actin remodeling, which facilitates RANK receptor clustering and enhances downstream pathways including NF-κB, MAPK, and JNK [120,125,134,165]. Rac1 promotes NF-κB signaling by activating IKK and accelerating IκBα degradation, enabling the nuclear translocation of NF-κB subunits and transcription of osteoclastogenic genes such as NFATc1, c-Fos, cathepsin K, and TRAP [141,142,166–169]. Genetic deletion or pharmacological inhibition of Rac1 impairs this transcriptional program and disrupts osteoclast maturation [123,132,169].

Rac1's role extends beyond differentiation into active bone resorption. It orchestrates the formation and maintenance of the ruffled border—the specialized membrane domain through which osteoclasts secrete protons and proteolytic enzymes to degrade mineralized matrix. Rac1 achieves this by coordinating actin polymerization with vesicle trafficking through effectors such as Wiskott-Aldrich syndrome protein (WASP), p21-activated kinase (PAK), and direct interactions with Rab7 [122,164,170,171]. Inactivation of Rac1 results in severe defects in sealing zone integrity and ruffled border assembly, leading to osteoclast dysfunction and osteopetrotic bone phenotypes, as observed in Rac-deficient models [121,132,172].

 doi:10.20944/preprints202604.1426.v1

Rac1 also regulates osteoclast motility and survival. Through its involvement in M-CSF receptor signaling, Rac1 activates PI3K/Akt pathways, enhancing cell survival and motility [173,174]. Additionally, guanine nucleotide exchange factors (GEFs) such as Dock5 and FARP2 are essential for Rac1 activation and localization to podosome cores, influencing cytoskeletal patterning and resorptive capacity [123,175–177].

Pathologically, Rac1 exacerbates inflammatory bone loss in conditions like rheumatoid arthritis and periodontitis. Inflammatory cytokines elevate Rac1 activity, enhancing osteoclastogenesis and bone resorption via RANKL amplification and cytoskeletal remodeling [133,178]. Moreover, Rac1 mediates reactive oxygen species (ROS) production, further amplifying pro-resorptive signaling in inflamed bone microenvironments [124,125,179]. This makes Rac1 a critical effector not only in physiological remodeling but also in pathological bone destruction [122,123].

Given its central role, Rac1 has emerged as a promising therapeutic target. Selective Rac1 inhibition—via genetic deletion, pharmacological blockers, or modulation of upstream regulators such as SRGAP2 and Drg2—has been shown to restrain osteoclast activation, reduce inflammatory bone loss, and improve bone strength in models of osteoporosis and arthritis [133,169,180,181]. Importantly, Rac1 inhibition can suppress osteoclast-driven bone resorption while preserving osteoblast function, offering a disease-modifying strategy with skeletal selectivity [121,122,181,182].

In conclusion, Rac1 integrates cytoskeletal remodeling, receptor signaling, and vesicular trafficking to control osteoclast differentiation and bone resorption. It is essential for the structural and functional competence of osteoclasts and plays a dual role in physiological bone remodeling and inflammatory bone diseases. Its targeting holds considerable translational promise for treating osteoporosis, rheumatoid arthritis, periodontitis, and other conditions characterized by excessive bone resorption.

3.2.3. Cdc42 in Osteoclastogenesis and Bone Resorption

Cdc42 is a central regulator of osteoclast differentiation, polarization, and bone-resorbing function. It orchestrates cytoskeletal remodeling, vesicle trafficking, and transcriptional activation to ensure osteoclast maturation and activity[120,122,123]. The importance of Cdc42 in skeletal homeostasis is underscored by evidence that both its loss and dysregulated activation can lead to severe bone disorders, including osteopetrosis and osteoporosis [115,126].

Osteoclast-specific deletion of Cdc42 impairs osteoclast differentiation and function, causes increased apoptosis, and leads to osteopetrosis marked by high bone mass and poor marrow cavity development due to suppressed resorption [115]. In contrast, global deletion of *Cdc42Gap*, a GTPase-activating protein that inactivates Cdc42, results in hyperactive osteoclastogenesis and osteoporosis-like bone loss [115]. This dichotomy reflects the need for finely tuned spatial and temporal control of Cdc42 signaling to maintain bone balance [120].

Cdc42 contributes to osteoclastogenesis by regulating RANKL- and M-CSF-dependent signaling cascades. It promotes cyclin D expression and retinoblastoma protein (Rb) phosphorylation to drive precursor proliferation [115,125]. It also enhances the expression and nuclear translocation of transcription factors like MITF and NFATc1, both essential for osteoclast-specific gene expression, including cathepsin K and TRAP [126,140,167].

Cdc42 governs the cytoskeletal architecture required for sealing zone and ruffled border formation. These actin-rich structures enable osteoclasts to adhere tightly to bone and create a specialized extracellular compartment for matrix degradation [121,182]. Cdc42 operates as part of the Par3/Par6/aPKC polarity complex to coordinate actin ring formation and apical-basal polarization [115,183]. Though not essential for initial actin ring formation, Cdc42 is required for its re-establishment during repolarization, highlighting its role in sustaining functional polarization under dynamic conditions [115,182].

The local activation of Cdc42 is regulated by guanine nucleotide exchange factors (GEFs) and GAPs. FGD6, a Cdc42-specific GEF, is essential for sealing zone organization and bone resorption. When phosphorylated by Src, FGD6 binds the effector IQGAP1 to facilitate actin polymerization at

 doi:10.20944/preprints202604.1426.v1

the resorption interface. In the absence of Src signaling, FGD6 preferentially interacts with the GAP Arhgap10, limiting Cdc42 activity and thereby restricting resorptive capacity [184]. This dynamic switch underscores the need for spatiotemporal precision in Cdc42 regulation [122].

Cdc42 also interacts with WASp/N-WASP to regulate Arp2/3-mediated actin nucleation. Although Cdc42 activation is necessary, it is not sufficient alone to activate WASp in osteoclasts; additional signals such as osteopontin are required to promote effective actin remodeling [123,136,171]. Furthermore, LC3, a microtubule-associated protein known for its role in autophagy, has been shown to regulate Cdc42-dependent actin ring formation, indicating cross-talk between the cytoskeletal and vesicular systems in bone resorption [183].

Genetic and pharmacological studies link Cdc42 to skeletal pathology. In osteoclasts, bisphosphonates inhibit protein prenylation, inadvertently causing sustained activation of Rho GTPases including Cdc42, which can impair cytoskeletal coordination and cell function [185,186]. SLIT2, a guidance cue, was found to suppress osteoclastogenesis by inhibiting Cdc42 activity, presenting a novel anti-resorptive mechanism [187]. Genome-wide association studies have identified Cdc42 as a susceptibility locus for osteoporosis; for instance, SNP rs6426749 increases Cdc42 expression by downregulating LINC00339 and is associated with lower bone mineral density [188]. **Cdc42 is also implicated in autosomal dominant osteopetrosis type II, where its hyperactivation—alongside Rac1 and MITF—contributes to dysregulated osteoclast activity and altered bone density** [126].

These findings position Cdc42 as a compelling therapeutic target. Its central role in actin regulation, vesicle fusion, and osteoclast transcriptional programming makes it a converging point for modulating bone resorption [120,121,182]. However, due to its ubiquitous function across cell types, systemic inhibition is not viable. Instead, osteoclast-specific approaches—such as RNAi, small-molecule inhibitors of FGD6-Cdc42 interaction, or agents that restore physiological cycling of Cdc42—hold greater translational promise [134,184]. For example, targeting Cdc42 in hyperresorptive conditions like rheumatoid arthritis or osteoporosis could dampen bone loss while preserving bone formation, especially when combined with anabolic treatments [122,123].

In summary, Cdc42 integrates extracellular signaling, cytoskeletal dynamics, and vesicular trafficking to coordinate osteoclast differentiation and bone resorption. Its dysregulation drives both osteopetrotic and osteoporotic phenotypes, depending on the direction and context of signaling imbalance [115,126,188]. As such, Cdc42 represents both a mechanistic linchpin in osteoclast biology and a promising node for precision intervention in bone-resorptive disorders [120,121,182].

3.3. Rho GTPases in Craniofacial Sutures

Craniofacial sutures are essential anatomical structures that function as growth sites and articulations between the bones of the skull. During postnatal development, these sutures remain unossified to accommodate the rapid expansion of the brain and allow proper cranial vault morphology. The maintenance of suture patency is governed by a balance between proliferation and differentiation of osteogenic precursors within the suture mesenchyme. Among the various molecular mechanisms that regulate this delicate balance, the Rho GTPases have emerged as key players due to their central roles in coordinating cytoskeletal architecture, mechanotransduction, and gene expression.

RhoA, Rac1, and Cdc42 are integral to craniofacial suture development, orchestrating cellular behaviors such as migration, polarity, adhesion, and differentiation. These small GTPases coordinate the dynamic processes that shape and maintain craniofacial sutures, which are critical for skull growth and form. Rac1 and Cdc42 are essential during early suture development, regulating cell migration and polarity [118,189]. Cdc42, in particular, is crucial for facial and palatal formation, influencing neural crest cell behavior and suture morphogenesis [67,189]. RhoA, on the other hand, plays a significant role in maintaining suture patency and regulating osteogenic differentiation within sutural mesenchymal cells [62,190].

 doi:10.20944/preprints202604.1426.v1

Collectively, RhoA, Rac1, and Cdc42 function in a coordinated manner to regulate the cellular dynamics of craniofacial sutures. Their activities ensure proper suture development, maintenance, and response to mechanical stimuli, which are crucial for normal craniofacial morphogenesis and function.

3.3.1. RhoA in Craniofacial Sutures

During cranial development, **RhoA signaling plays a central role at the osteogenic fronts**, where it regulates cytoskeletal remodeling critical for the alignment, migration, and differentiation of osteoblasts along the advancing cranial bone edges. Experimental studies in murine models demonstrate that constitutive activation of RhoA enhances osteoblast differentiation, elevates the expression of osteogenic transcription factors such as Runx2 and Sp7, and leads to premature suture fusion, while RhoA inhibition delays ossification and maintains suture patency [58,62,87,190].

RhoA functions as a molecular transducer of mechanical cues generated by brain expansion, linking extracellular force with intracellular responses via integrins and focal adhesions. In this context, RhoA activation drives actomyosin contractility and cytoskeletal tension, which in turn regulate the nuclear localization and transcriptional activity of YAP/TAZ, key effectors of mechanosensitive osteogenesis [42,62,190–192]. For example, in sagittal suture mesenchymal stem cells (SSCs), mechanical tension-induced osteogenic differentiation depends on the RhoA–ROCK–TAZ axis [190].

The mechanotransductive role of RhoA is tightly coupled with major developmental pathways. Wnt/β-catenin signaling, a known promoter of osteogenesis, is modulated through cytoskeletal dynamics influenced by RhoA activity [193]. Similarly, RhoA intersects with FGF, TGF-β/BMP, and PI3K/AKT signaling cascades, integrating both biochemical and mechanical pro-osteogenic signals [62,193–197].

Importantly, **RhoA is implicated in the pathogenesis of craniosynostosis**, a disorder characterized by premature suture fusion. Human suture specimens and animal models of nonsyndromic sagittal craniosynostosis show upregulation of RHOA and associated genes like DAAM1 in fusing sutures, indicating enhanced integrin–ECM interactions and osteogenic activation [198]. Genetic studies further highlight RhoA dysregulation downstream of syndromic mutations, such as those in FGFR2, which activate ERK-MAPK and PI3K-AKT pathways and are known to potentiate RhoA signaling [195–197]. Moreover, microarray analysis in osteoblasts expressing the FGFR2 Ser252Trp Apert mutation revealed increased RhoA expression alongside elevated PKCα and IL-1α, suggesting inflammatory and kinase-mediated regulation of RhoA in craniosynostotic conditions [199].

Pharmacological inhibition of RhoA effectors offers translational potential: **ROCK inhibitors can delay or prevent suture fusion** in ex vivo calvarial cultures, supporting the feasibility of targeting the RhoA pathway in non-surgical treatment strategies for craniosynostosis [190,198].

Furthermore, **RhoA activity is modulated by genetic regulators and scaffolds critical for cranial ossification**. For instance, Nf2 deletion in mesenchymal stem cells impairs cranial bone regeneration and is associated with diminished RhoA activity, highlighting its necessity in osteoprogenitor function [200]. The IRF6–ARHGAP29–RhoA axis also contributes to craniofacial development, where GAP-mediated regulation of RhoA activity ensures appropriate epithelial and mesenchymal interactions during morphogenesis [201].

Tensile loading of cranial sutures triggers upregulation of RhoA-associated vascular and osteogenic genes, including Vegfa and Mmp2, suggesting that RhoA not only regulates osteoblast behavior but also coordinates angiogenic support during mechanical adaptation of sutures [202].

Despite its well-established roles, **several critical questions remain**, particularly regarding the spatiotemporal regulation of RhoA in different suture cell populations. The interplay between RhoA and other small GTPases such as Rac1 and Cdc42—which are also essential for neural crest cell proliferation and craniofacial patterning—needs to be dissected at the single-cell level to uncover potential redundancy or synergy [67,118,189]. Emerging technologies such as single-cell RNA

 doi:10.20944/preprints202604.1426.v1

sequencing, lineage tracing, and live imaging will be instrumental in defining RhoA-responsive subpopulations and their dynamics during suture development.

In conclusion, **RhoA serves as an essential integrator of genetic, mechanical, and biochemical signals during cranial suture morphogenesis**, and its dysregulation contributes to craniosynostosis through aberrant osteogenic activation. While therapeutic modulation of this pathway holds promise, strategies must be tailored to achieve spatial and temporal precision due to the ubiquitous nature of RhoA across tissues.

3.3.2. Rac1 in Craniofacial Sutures

Rac1 plays a pivotal role in cranial suture biology through its regulation of cranial neural crest cells (NCCs), which are indispensable for craniofacial development. Conditional deletion of Rac1 specifically in NCCs results in profound craniofacial malformations, including ectodermal detachment, mesenchymal loss, and craniofacial clefts as early as embryonic day 12, emphasizing Rac1's role in coordinating cell-matrix interactions and maintaining tissue integrity during cranial morphogenesis [203].

At the cellular level, Rac1 orchestrates adhesion, migration, and proliferation of both NCCs and sutural mesenchymal cells, processes essential for suture patency and skull growth. Rac1-deficient NCCs display impaired lamellipodia formation and reduced responsiveness to extracellular matrix signals, leading to defective colonization of craniofacial primordia and disorganized suture structure [203,204]. The disruption of Rac1 in the mechanosensitive mesenchyme of the scalp and cranial sutures also leads to failure in mesenchymal condensation and organization, highlighting its importance in translating biomechanical cues into developmental outcomes [204].

Rac1 enables NCCs and their derivatives to respond to mitogenic cues, such as EGF, in a temporally restricted manner, thereby modulating cell proliferation and lineage decisions during cranial base and midface development. Hyperactive or dysregulated Rac1 activity results in abnormal proliferation, impaired migration, and differentiation defects, particularly in the midbrain and frontonasal regions [205].

Mechanistically, Rac1 functions through complex signaling networks, interacting with effectors such as Trio, a guanine nucleotide exchange factor (GEF) critical for Rac1 activation, and Myh9, a cytoskeletal regulator. Trio-Rac1 signaling is required for normal actin cytoskeleton organization, nuclear positioning, and gene expression in NCC-derived cranial mesenchyme [206]. In addition, Rac1 modulates PDGFRα-mediated signaling and cytoskeletal rearrangements in the medial nasal process, a key structure in primary palate formation [207].

Transcriptomic and genetic studies support the involvement of Rac1 and its pathway components in human craniofacial disorders. Copy number variations (CNVs) affecting genes in the Rac1 signaling axis, including those encoding GEFs and cytoskeletal proteins, are enriched in patients with orofacial clefts and other craniofacial anomalies [Conte et al., 2016]. Moreover, Rac1 activity intersects with cellular boundary formation processes that ensure spatial compartmentalization in cranial mesenchyme, further reinforcing its role in maintaining the architecture of the cranial vault and preventing pathological suture fusion [208].

In cranial sutures specifically, Rac1 is likely to maintain the stem/progenitor pool and control their interactions with the surrounding extracellular matrix, thereby preventing premature ossification. Although the direct evidence for Rac1 in postnatal suture biology is emerging, its function in NCC-derived cranial progenitors suggests that impaired Rac1 activity could predispose to craniosynostosis by disrupting cellular behaviors essential for suture maintenance [118,203].

Importantly, Rac1 functions in coordination with other small Rho GTPases, particularly Cdc42 and RhoA, to regulate actin dynamics and transcriptional responses. During early craniofacial development, Rac1 and Cdc42 exhibit stage-specific control over NCC proliferation, influencing cranial shape and volume [118]. Rac1 also interfaces with transcriptional programs by regulating SRF (serum response factor) and downstream targets that couple cytoskeletal status to gene expression in craniofacial mesenchyme [204].

 doi:10.20944/preprints202604.1426.v1

In summary, Rac1 is a central coordinator of craniofacial morphogenesis, acting through neural crest regulation, cytoskeletal dynamics, and signal integration. Its functions are critical in maintaining sutural mesenchyme organization, guiding NCC behavior, and preventing aberrant suture fusion. Deciphering the Rac1-mediated molecular framework offers a promising avenue for understanding and potentially treating craniofacial disorders such as craniosynostosis, orofacial clefting, and midline defects.

3.3.3. Cdc42 in Craniofacial Sutures

Cdc42 is a critical regulator of cranial suture morphogenesis, acting through its control of neural crest cell (NCC) behavior, cytoskeletal remodeling, and ossification dynamics. Conditional knockout of *Cdc42* in NCCs leads to profound craniofacial abnormalities, including cleft lip, shortened snout, and unfused nasal capsules, all of which reflect disrupted cranial suture patterning [67,189]. These defects stem from impaired NCC migration, loss of polarity, and failure of actin cytoskeletal organization—processes for which Cdc42 is indispensable [189].

Further analysis reveals that **Cdc42 governs palatogenesis and cranial ossification through its influence on palatal shelf elevation and fusion**. Inactivation of *Cdc42* in NCCs results in cleft palate due to defective elevation of the palatal shelves and insufficient midline fusion. Skeletal preparations of mutant embryos show marked hypoplasia and reduced mineralization in frontal, nasal, and premaxillary bones, highlighting the essential role of Cdc42 in suture-associated bone development [67,209].

At the molecular level, Cdc42 interacts with the effector protein Cdc42EP1, which is required for the directional migration of NCCs and cranial cartilage development. Cdc42 regulates the cortical and cytoplasmic localization of Cdc42EP1 to maintain proper cell motility. Disruption of this signaling axis causes loss or malformation of cranial cartilages and may impair suture mesenchyme integrity [210]. This interaction underscores how Cdc42 governs not only the behavior of individual cells but also the coordinated morphogenesis of skeletal elements derived from cranial NCCs.

Cdc42 is also directly involved in the ossification and shaping of cranial sutures. Its inactivation in cranial mesenchymal tissues results in delayed suture closure, reduced bone bridging, and thinner calvarial bone plates, indicating a role in suture patency and mineral deposition. Histological and molecular studies demonstrate that Cdc42 is required for the recruitment and differentiation of osteoprogenitors in suture regions, likely through its impact on actin cytoskeleton dynamics and transcriptional programs [209].

Human genetic studies further confirm the critical role of CDC42 in craniofacial development. Mutations or functional dysregulation of *CDC42* in patients result in diverse craniofacial malformations, including hypertelorism, midface hypoplasia, and cleft palate, sometimes associated with *CDC42*-related syndromes such as Takenouchi-Kosaki syndrome [211,212]. These mutations often affect GTP binding or hydrolysis domains of CDC42, impairing its ability to regulate downstream effectors required for morphogenesis.

Mechanistically, Cdc42 operates through signaling pathways and cytoskeletal regulators that interface with actomyosin contractility and extracellular matrix remodeling. It functions in parallel with other small GTPases like Rac1 and RhoA to coordinate NCC proliferation and craniofacial patterning [118,206]. Cdc42 also cooperates with Trio and Myh9 to influence the cytoskeletal architecture of neural crest-derived mesenchyme, ensuring proper facial structure and cranial bone formation [206].

In summary, Cdc42 is indispensable for cranial suture development, integrating cytoskeletal dynamics, NCC migration, and osteogenic signaling. Its inactivation leads to both structural and molecular defects that culminate in craniofacial anomalies, including clefts, cartilage dysplasia, and suture hypoplasia. Given its central role, Cdc42 represents a potential therapeutic target for modulating craniofacial development and preventing pathological suture fusion.

 doi:10.20944/preprints202604.1426.v1

4. Rho GTPases in Odontogenesis

4.1. Rho GTPases in Early Stage of Tooth Development

Tooth development is a complex process involving reciprocal epithelial-mesenchymal interactions, tightly regulated spatial and temporal gene expression, and coordinated cell behaviors such as proliferation, migration, shape change, and differentiation. RhoA, Rac1, and Cdc42 are pivotal regulators during the early stages of tooth development, encompassing the initiation, bud, cap, and early bell stages. These small GTPases orchestrate cellular behaviors such as proliferation, migration, polarity, and epithelial-mesenchymal interactions, which are essential for the formation of the tooth germ and the establishment of the dental lamina.

At the onset of tooth development around embryonic day (E) 11.5, RhoA, Rac1, and Cdc42 are expressed in the dental epithelium and mesenchyme [213,214]. Their expression patterns are dynamic, with RhoA and Rac1 showing increased expression during the bud and cap stages, while Cdc42 expression becomes prominent in the inner and outer enamel epithelia during the cap stage. This spatiotemporal expression suggests their roles in epithelial morphogenesis and the establishment of polarity within the developing tooth structures.

Functional studies have demonstrated that these GTPases are critical for proper tooth development. For instance, inhibition of RhoA, Rac1, or Cdc42 during early tooth development disrupts cell proliferation and migration, leading to defects in tooth morphogenesis [18,213,215,216]. These GTPases are involved in regulating epithelial-mesenchymal interactions, which are crucial for the reciprocal signaling between the dental epithelium and mesenchyme that drives tooth development.

4.1.1. RhoA in Early Stage of Tooth Development

RhoA is emerging as a key intracellular signaling mediator in the morphogenetic events at early stages of tooth development, particularly due to its ability to link extracellular cues with cytoskeletal organization and mechanical force generation.

During the **placode, bud, and early cap stages,** RhoA is expressed in both dental epithelial and mesenchymal tissues, with dynamic spatial patterns [214,215]. It is particularly active in regions undergoing shape change, such as the invaginating epithelium and the condensing mesenchyme. RhoA functions mainly by regulating **actin cytoskeleton remodeling** and **cell-cell junctions**, which are essential for epithelial bending, invagination, and basal constriction [7,121,217]. In the dental epithelium, RhoA promotes apical actomyosin contractility through its downstream effector **ROCK,** contributing to the tissue folding that forms the epithelial bud [218–220]. Experimental disruption of RhoA activity (e.g., by dominant-negative mutants or ROCK inhibitors) results in impaired epithelial invagination, reduced apical constriction, and disrupted bud morphology [221,222]. These findings suggest that RhoA activity may be necessary for coordinating the mechanical forces that shape the early tooth germ.

In the underlying mesenchyme, RhoA is involved in cell condensation, a hallmark of mesenchymal preparation for odontogenic signaling. Neural crest-derived mesenchymal cells migrate toward the epithelial signaling center and condense around the bud, guided by growth factors such as FGF8 and BMP4 [223]. RhoA regulates mesenchymal cell motility and adhesion by controlling integrin-mediated attachment and the organization of actin stress fibers [224]. By promoting focal adhesion assembly and cytoskeletal stiffness, RhoA helps establish a mechanically competent mesenchymal environment that can receive and respond to epithelial signals, further stabilizing the epithelial-mesenchymal interface [224,225].

Moreover, RhoA plays a role in the **regulation of gene expression** associated with odontogenic fate. Mechanical signals transduced through RhoA-ROCK pathways can influence the nuclear localization and activity of transcriptional coactivators such as **YAP/TAZ**, which are known to modulate cell proliferation and organ size [22,61,192,226,227]. In tooth development, YAP/TAZ are expressed in both dental epithelium and mesenchyme, and their activity is at least partially

 doi:10.20944/preprints202604.1426.v1

dependent on RhoA-mediated cytoskeletal tension [227–229]. This suggests that RhoA is not only important for morphogenetic movements but also for integrating biomechanical cues into developmental gene regulatory networks.

There is also evidence of **crosstalk between RhoA signaling and classical developmental pathways** such as **Wnt/β-catenin, BMP, and FGF**, which are critical in tooth initiation and patterning [11,215,230,231]. RhoA may act downstream or in parallel to these pathways to regulate cell shape and movement. For instance, Wnt signaling, essential for tooth induction, can enhance RhoA activity [11,232], while BMPs may influence RhoA through non-canonical SMAD-independent mechanisms [233,234].

In sum, RhoA is a central player in the early stages of tooth development. It regulates key cellular processes such as epithelial bending, mesenchymal condensation, and the integration of mechanical and biochemical signals. Its function is vital for shaping the tooth germ and establishing the foundation for later stages of differentiation and morphogenesis. Disruption of RhoA signaling leads to profound defects in early tooth morphogenesis, underscoring its indispensable role in craniofacial organogenesis.

4.1.2. Rac1 in Early Stage of Tooth Development

In the embryonic stages of tooth morphogenesis, Rac1 plays a key role in shaping epithelial cell behavior and coordinating epithelial-mesenchymal interactions, which are vital for the formation of tooth structures. During the initiation of tooth development, Rac1 is active in the oral epithelium, where it regulates the cellular rearrangements required for the formation of the dental placode [214,215]. The dental placode is the first sign of tooth development, and its formation involves the thickening of the epithelium in response to signaling from the underlying mesenchyme. Rac1 mediates actin polymerization and rearrangement in the epithelial cells, facilitating the cell shape changes necessary for the invagination of the epithelium into the mesenchyme to form the tooth bud. This epithelial invagination is an early and essential step in tooth morphogenesis, and Rac1 is critical in regulating the actin cytoskeleton during this process.

As the tooth develops from the bud stage to the cap stage, Rac1 continues to regulate cytoskeletal dynamics, which are necessary for epithelial-mesenchymal interactions. The transition from the dental bud to the dental cap involves complex cellular behaviors, including epithelial proliferation, folding, and cellular rearrangements. Rac1's ability to modulate actin filament dynamics and cell-cell adhesion ensures the integrity of the epithelial layer during these transitions [216,235]. Furthermore, Rac1 is essential for the coordinated migration of epithelial cells as they begin to form the dental lamina and the tooth bud expands [236].

At the molecular level, Rac1 signaling is tightly connected to several developmental pathways that regulate early tooth morphogenesis. For example, Rac1 has been shown to interact with the Wnt/β-catenin signaling pathway [11,28,86,88], which is critical for tooth patterning and epithelial-mesenchymal signaling. Rac1 also regulates signaling pathways involved in cell adhesion, such as integrin signaling [64,103,237], ensuring that epithelial cells remain properly attached to the basement membrane during the transition between tooth bud and cap stages.

Studies have highlighted the importance of Rac1 in regulating the actomyosin contractility that drives the mechanical forces necessary for epithelial folding and invagination during early tooth development [221,238]. By controlling these mechanical forces, Rac1 ensures that the developing tooth bud maintains its proper structure and continues to grow in the correct direction.

4.1.3. Cdc42 in Early Stage of Tooth Development

Cdc42 plays a pivotal role in the early stages of tooth development, particularly in the formation and differentiation of the enamel organ. Its functions encompass regulating cytoskeletal dynamics, cell polarity, and signaling pathways essential for proper tooth morphogenesis.

 doi:10.20944/preprints202604.1426.v1

During early tooth development, Cdc42 is prominently expressed in the dental epithelium, including the inner enamel epithelium (IEE) and the stratum intermedium (SI). This expression pattern suggests its involvement in epithelial cell organization and function [213].

4.2. Rho GTPases in Dentinogenesis

The process of dentin formation carried out by odontoblasts—highly specialized cells derived from cranial neural crest-origin mesenchymal progenitors. Dentinogenesis occurs in two main phases: the initial formation of predentin, an unmineralized matrix rich in collagen and non-collagenous proteins, followed by its mineralization into mature dentin. RhoA, Rac1, and Cdc42 are critical regulators of dentinogenesis through their control of odontoblast differentiation, polarization, cytoskeletal organization, and matrix secretion. These small GTPases coordinate the morphological and functional transformation of dental mesenchymal cells into polarized, columnar odontoblasts that produce the dentin matrix [239].

RhoA plays a central role in cytoskeletal remodeling during early odontoblast differentiation. It promotes actin stress fiber formation and focal adhesion assembly via the RhoA-ROCK pathway, enabling cell elongation and alignment along the basement membrane [239,240]. RhoA also facilitates odontoblast polarization, ensuring that secretion of dentin matrix proteins—such as type I collagen, dentin sialophosphoprotein (DSPP), and dentin matrix protein 1 (DMP1)—occurs from the apical end of the odontoblast process into the predentin layer [214].

Rac1 contributes to odontoblast polarity and survival, regulating lamellipodia formation and cell–matrix adhesion. It is also involved in maintaining microtubule organization, crucial for directional secretion [243,244]. Inhibition of Rac1 impairs odontoblast morphology, reduces expression of odontogenic markers, and results in hypomineralized or disorganized dentin [214,216].

Cdc42 is essential for the establishment of apical-basal polarity and vesicle trafficking in odontoblasts. It regulates filopodia formation and Golgi positioning, which are important for targeted delivery of matrix components [32,239,241]. Cdc42 also participates in cell–cell junction stability, ensuring the coordinated activity of adjacent odontoblasts [241,242].

All three GTPases modulate mechanotransduction pathways, such as YAP/TAZ signaling, which link cytoskeletal tension to transcriptional activation of odontogenic genes. Disruption of their activity in experimental models leads to defective dentin formation, indicating their indispensable roles [214,216,242,243]. Together, RhoA, Rac1, and Cdc42 integrate mechanical and biochemical cues to orchestrate the cellular architecture and functional output required for normal dentinogenesis.

4.2.1. RhoA in Dentinogenesis

RhoA is essential at multiple points in this process, primarily through its regulation of cytoskeletal dynamics, cell polarity, migration, adhesion, and gene expression. These actions are especially important during odontoblast differentiation, polarization, and the directional secretion of dentin matrix components.

During early odontoblast differentiation, mesenchymal stem cells in the dental papilla proliferate and migrate toward the basement membrane separating them from the dental epithelium. RhoA regulates the actin cytoskeleton and cell-matrix interactions, enabling proper alignment and elongation of pre-odontoblasts [241,244]. Activation of RhoA promotes stress fiber formation and focal adhesion assembly, primarily through the RhoA-ROCK signaling pathway [56,239,245]. These cytoskeletal structures are necessary for the transformation of undifferentiated dental mesenchymal cells into polarized, columnar odontoblasts that line the outer surface of the dental pulp.

A critical feature of RhoA's role in dentinogenesis is its influence on cell polarity and secretion orientation. Odontoblasts secrete dentin matrix components such as type I collagen, DSPP, and DMP1 in a highly polarized manner from their apical ends into the forming predentin layer. RhoA regulates this polarized secretory function by coordinating actin filament dynamics and stabilizing microtubules in the odontoblast process [239,241], a long cellular extension that projects into the dentin. Experimental inhibition of RhoA or ROCK in odontoblasts leads to defects in cell polarity and

 doi:10.20944/preprints202604.1426.v1

morphology, disrupted secretion, and irregular dentin structure, supporting its centrality in functional odontoblast architecture [240,241,246,247].

In addition to structural regulation, RhoA influences the transcriptional regulation of odontogenic genes. It is involved in mechanical signal transduction through the cytoskeleton, leading to nuclear activation of transcription coactivators such as YAP and TAZ [62,248]. These mechanosensitive proteins can interact with transcription factors including Runx2 and Sp7 to modulate the expression of genes critical for odontoblast differentiation and matrix mineralization [240,249,250]. Furthermore, there is evidence of crosstalk between RhoA and key developmental pathways such as BMP, TGF-β, and Wnt, which are known to regulate odontogenesis [230,244,245,251–253]. For instance, BMP-induced odontoblastic differentiation is enhanced by RhoA-mediated cytoskeletal organization, which may stabilize SMAD signaling and improve downstream transcriptional responses [239].

Although direct evidence is limited, RhoA is believed to play a supportive role in dentin mineralization during the late stages of dentinogenesis. Following the secretion of the collagen-rich predentin matrix by odontoblasts, mineralization proceeds through the deposition of hydroxyapatite crystals. RhoA, known for its regulation of cytoskeletal dynamics and vesicle trafficking in various mineralizing cells, may contribute to this process by influencing the secretion of matrix vesicles [254]—small extracellular organelles involved in initiating mineral nucleation. Furthermore, by modulating actin organization and vesicle transport, RhoA is thought to facilitate the targeted delivery of mineralization-related enzymes such as alkaline phosphatase and matrix metalloproteinases to the mineralization front, thus supporting efficient and localized mineral deposition [255].

Dysregulation of RhoA activity has been implicated in dental pathologies. Overactivation of RhoA/ROCK signaling can lead to excessive actomyosin contraction, which may cause apoptosis or disrupt odontoblast differentiation. Conversely, insufficient RhoA activity compromises cytoskeletal integrity and cell polarity, both of which are essential for proper dentin formation and mineralization [240,241]. Although direct evidence from transgenic models with odontoblast-specific RhoA inhibition is currently limited, disruptions in cytoskeletal dynamics are known to impair odontoblast elongation and polarity, which can negatively affect the expression of key dentin matrix proteins such as DSPP and DMP1, potentially resulting in a disorganized dentin matrix.

Overall, RhoA is a central intracellular regulator of dentinogenesis, guiding the cytoskeletal changes, polarity establishment, migration, and matrix secretion that define odontoblast function. Its ability to integrate mechanical and biochemical signals makes it indispensable in shaping the structure and mineralization of dentin. Future studies may explore RhoA as a therapeutic target for enhancing reparative dentinogenesis or treating dentin defects in genetic and acquired dental diseases.

4.2.2. Rac1 in Dentinogenesis

Rac1 plays a critical role in dentinogenesis by regulating odontoblast differentiation, cytoskeletal organization, and polarized secretion of dentin matrix components. As mesenchymal cells from the dental papilla differentiate into odontoblasts, they undergo significant changes in morphology, alignment, and polarity, all of which are tightly controlled by Rac1-mediated signaling. Rac1 is activated in pre-odontoblasts during their migration and alignment along the basement membrane adjacent to the inner enamel epithelium. Through modulation of actin filament dynamics and cell-matrix adhesion, Rac1 facilitates the elongation and polarization of odontoblasts, which is necessary for their secretory activity [214,239,241]. These structural adaptations may be coordinated through Rac1's downstream effectors such as WAVE and PAK, which regulate lamellipodia formation and microtubule stabilization [25,256,257].

Once odontoblasts polarize, Rac1 contributes to the vesicular transport and apical secretion of key dentin matrix proteins including type I collagen, DSPP, and DMP1. Disruption of Rac1 function impairs this polarized secretion, leading to discontinuous predentin deposition and defective tubular

 doi:10.20944/preprints202604.1426.v1

dentin structure [214,258]. Recent studies, including conditional knockout models targeting Rac1 in neural crest-derived mesenchyme, show that Rac1-deficient odontoblasts exhibit abnormal morphology, lose their apical-basal polarity, and fail to maintain organized dentinogenesis. These defects are associated with a decrease in DSPP expression and hypomineralized dentin.

Recent studies have expanded the understanding of Rac1's function by linking it to several key signaling pathways that govern odontogenesis. Rac1 has been implicated in modulating Wnt/β-catenin signaling, which is known to be crucial for odontoblast differentiation and dentin matrix gene expression [86,88,259]. Moreover, Rac1 activity influences the TGF-β/Smad and MAPK pathways, both of which are vital in odontogenic induction and matrix synthesis [252,260]. Through these interactions, Rac1 serves as a convergence point for extracellular cues that direct the transcriptional programming of odontoblasts.

While Rac1's general roles in cell migration and differentiation suggest it may contribute to dental tissue repair and regeneration, especially in injury models, specific studies on Rac1 in reparative dentinogenesis or regenerative endodontics are currently lacking. Thus, Rac1 is recognized as a key intracellular regulator in primary dentinogenesis, with its broader potential in dental regeneration remaining an open area for further investigation. Its diverse roles at multiple stages of odontoblast maturation make it an attractive target for therapeutic modulation in dental tissue engineering and regenerative dentistry.

4.2.3. Cdc42 in Dentinogenesis

Cdc42 plays a crucial role in odontoblast function and dentinogenesis. It regulates cytoskeletal organization and cell polarity, essential for odontoblast differentiation and dentin formation. Cdc42 is indispensable for the formation and elongation of odontoblast processes, which are vital for dentin secretion and the establishment of dentinal tubules [246]. Disruption of Cdc42 activity leads to impaired odontoblast morphology and defective dentin structure [242,246].

One key pathway involving Cdc42 is the WNT5a-ROR2-CDC42-JNK signaling axis [260]. This non-canonical Wnt pathway modulates actin cytoskeleton reorganization, facilitating odontoblast polarization. Activation of this pathway promotes the alignment of odontoblasts and the formation of dentinal tubules, which are critical for proper dentin deposition. Studies have shown that Wnt5a binds to the Ror2 receptor, leading to the activation of Cdc42 and downstream JNK signaling, resulting in cytoskeletal rearrangements necessary for cell polarization [244,260].

Additionally, Cdc42 is implicated in the regulation of the PAR polarity complex (PAR3-PAR6-aPKC), which contributes to establishing apical-basal polarity in odontoblasts [261]. This polarity is essential for the directional secretion of dentin matrix components and the maintenance of organized tissue architecture.

In the context of tooth root development, Cdc42 has been shown to influence the proliferation of dental mesenchymal cells. Activation of Cdc42 can restore cell proliferation and partially rescue root development defects in models with impaired Ror2 signaling, highlighting its role in root morphogenesis [242,262].

4.3. Rho GTPases in Amelogenesis

Amelogenesis is the multistage process by which enamel is formed by specialized epithelial cells called ameloblasts. It begins with the **pre-secretory stage**, where inner enamel epithelial cells differentiate into polarized pre-ameloblasts that prepare for protein secretion. During the **secretory stage**, ameloblasts develop a specialized apical extension called **Tome's process**, which guides the organized secretion of enamel matrix proteins, shaping the characteristic prism pattern of enamel. This is followed by the **maturation stage**, in which ameloblasts alternate between ruffle-ended and smooth-ended phases to remove matrix proteins and facilitate full mineralization of enamel crystals.

RhoA, Rac1, and Cdc42 orchestrate cytoskeletal dynamics, polarity, and vesicle trafficking in ameloblasts, making them essential regulators of amelogenesis. During the pre-secretory stage, these GTPases contribute to the elongation and polarization of pre-ameloblasts, enabling the transition

 doi:10.20944/preprints202604.1426.v1

from cuboidal to columnar morphology, a prerequisite for directional enamel matrix secretion [17]. RhoA, primarily via its effector ROCK, promotes actin stress fiber formation and focal adhesion, anchoring ameloblasts to the basement membrane and establishing apical-basal polarity [17,263].

In the secretory stage, Rac1 and Cdc42 coordinate the formation and maintenance of the Tomes' process [17], an apical cellular extension essential for the secretion of enamel matrix proteins such as amelogenin and enamelin. Rac1 regulates actin polymerization necessary for vesicle trafficking, while Cdc42 is involved in establishing planar cell polarity and maintaining tight junctions [264], which ensure synchronized secretion across ameloblasts. Disruption of either GTPase impairs enamel protein secretion and leads to defective prism architecture [17,213,216].

During maturation, RhoA and Cdc42 contribute to the cyclical modulation between ruffle-ended and smooth-ended ameloblasts, supporting enamel mineralization by regulating ion transport and resorptive activity [263–265]. These GTPases also influence mechanotransduction pathways such as YAP/TAZ and interact with signaling cascades like Wnt and Semaphorin 4D-Plexin-B1, integrating extracellular cues with intracellular structural changes [266–268].

Altogether, RhoA, Rac1, and Cdc42 coordinate cytoskeletal architecture, vesicular dynamics, and intercellular junctions to ensure precise enamel formation, and their dysregulation is associated with enamel pathologies such as amelogenesis imperfecta.

4.3.1. RhoA in Amelogenesis

RhoA plays a pivotal role in regulating the cytoskeletal dynamics, polarity, and secretory function of ameloblasts throughout the various stages of their differentiation. Its activity is essential from the initial polarization of ameloblast precursors to the complex regulation of enamel matrix secretion and mineralization.

During the pre-secretory stage, inner enamel epithelial cells differentiate into pre-ameloblasts in response to inductive cues from the underlying dental papilla. At this stage, RhoA is crucial for initiating cytoskeletal reorganization and cell elongation, facilitating the transformation of cells from a cuboidal to a polarized columnar morphology [17,263,265,268]. This shift is a prerequisite for the directional secretion of enamel matrix proteins. Acting through its canonical effector, ROCK, RhoA promotes the formation of actin stress fibers and focal adhesions that anchor cells to the basement membrane and help establish apical-basal polarity. Disruption of RhoA or ROCK during this period leads to malformed and poorly organized ameloblasts incapable of sustaining enamel formation [17,265,269].

As ameloblasts enter the secretory stage, they develop a distinctive apical extension known as Tomes' process, which is essential for the directional secretion of critical enamel proteins such as amelogenin, ameloblastin, and enamelin. RhoA maintains the structural and functional integrity of this highly polarized architecture. It regulates the organization of apical actin filaments and coordinates vesicle trafficking necessary for precise exocytosis [270]. In addition to its role in secretion, RhoA signaling supports the formation and maintenance of adherens and tight junctions between ameloblasts, ensuring synchronized function and epithelial barrier integrity during enamel deposition [271]. RhoA also functions as a key downstream effector integrating signals from BMP and Wnt pathways to regulate ameloblast polarization and enamel formation. For instance, BMP-induced Smurf1 mediates ubiquitination and degradation of RhoA, controlling cytoskeletal dynamics and cell polarity [272], while Wnt signaling activates RhoA to promote actin remodeling and secretory activity [232].

In the transitional and maturation stages, ameloblasts shorten and adopt dynamic morphologies that alternate between ruffle-ended and smooth-ended forms. These morphological changes facilitate cycles of protein resorption and ion transport essential for enamel mineralization. RhoA contributes to this cellular plasticity by regulating actomyosin contractility and cytoskeletal remodeling [273].

Recent studies have identified the Semaphorin 4D–RhoA–Akt pathway as a key regulatory axis in amelogenesis [268]. Semaphorin 4D, through its receptor Plexin-B1, activates RhoA to influence cytoskeletal arrangement and vesicular dynamics. This pathway has been shown to modulate

 doi:10.20944/preprints202604.1426.v1

ameloblast polarity and enamel matrix secretion. Disruption of this cascade leads to misaligned ameloblasts and defective enamel structure, reinforcing RhoA's role as an integrator of external morphogenetic signals with intracellular architectural control. Additionally, ameloblasts experience dynamic changes in extracellular matrix (ECM) composition and stiffness during enamel development. RhoA enables these cells to respond to such mechanical cues, coordinating cytoskeletal reorganization and secretory activity with the evolving physical environment [17,239,265]. It also facilitates membrane recycling and vesicular trafficking to accommodate the extensive protein secretion required during the secretory phase [239,254].

Experimental models have further underscored RhoA's importance in enamel development. Dominant-negative RhoA in ameloblasts results in severe enamel defects, including hypoplasia, disrupted prism organization, and reduced mineral density [265,274]. These defects are accompanied by mislocalization of junctional proteins such as E-cadherin and ZO-1, indicating compromised epithelial cohesion and transport regulation.

In summary, RhoA is indispensable for all stages of amelogenesis. It governs cytoskeletal remodeling necessary for cell elongation and polarization, orchestrates the targeted secretion of enamel matrix proteins, stabilizes epithelial junctions, and mediates mechanosensitive signaling pathways required for matrix processing and mineral deposition. By integrating mechanical cues and developmental signals, RhoA enables ameloblasts to perform their complex functions with spatial and temporal precision. Dysregulation of RhoA signaling contributes to enamel pathologies such as amelogenesis imperfecta, positioning it as a key target in the study and potential treatment of enamel developmental disorders.

4.3.2. Rac1 in Amelogenesis

Rac1 plays a crucial role in amelogenesis by regulating key cellular processes during the differentiation of ameloblasts. During the early stages of ameloblast differentiation, Rac1 expression is upregulated. It localizes predominantly at the distal pole of polarizing ameloblasts, where it exhibits a punctate distribution pattern [214]. This localization is associated with the formation of cellular protrusions and the establishment of cell polarity. Rac1 also regulates laminin-10/-11, interacts with integrin $\alpha 6\beta 4$, and mediate cell polarity, spreading, and filopodia formation of the dental epithelium [17,275]. These interactions are crucial for establishing epithelial cell behavior during the early stages of ameloblast differentiation.

Functional studies utilizing epithelial-specific Rac1 knockout mice have provided insights into the role of Rac1 in amelogenesis [216]. These mice exhibit defects in enamel formation, characterized by a complete loss of enamel upon tooth eruption. Histological analyses reveal that the Tomes' processes of Rac1-deficient ameloblasts lose contact with the forming enamel matrix in unerupted teeth. Additionally, the expression levels of key enamel matrix proteins, such as amelogenin and ameloblastin, are significantly reduced in these ameloblasts. These findings underscore the importance of Rac1 in mediating cell-matrix interactions and facilitating matrix biomineralization during enamel formation.

4.3.3. Cdc42 in Amelogenesis

Cdc42 is crucial for proper enamel formation through its regulation of cytoskeletal dynamics, cell polarity, and cellular metabolism during amelogenesis. Studies using epithelial-specific Cdc42 knockout mouse models have revealed that loss of Cdc42 disrupts the architecture of the enamel organ, leading to defects such as impaired ameloblast polarization, compromised cell-cell adhesion, and even odontogenic cyst formation, underscoring its role in maintaining epithelial integrity during tooth development [213]. Further research has shown that deletion of Cdc42 in dental epithelium causes mitochondrial dysfunction and increases reactive oxygen species production, resulting in oxidative stress that hinders ameloblast differentiation and leads to hypomineralized enamel, highlighting Cdc42's involvement in regulating mitochondrial health and redox homeostasis necessary for enamel matrix secretion [18]. Additionally, conditional knockout of Cdc42 during the

 doi:10.20944/preprints202604.1426.v1

maturation phase of amelogenesis causes enamel hypermaturation characterized by abnormal prismatic structure and defective enamel protein resorption, suggesting that Cdc42 also modulates enamel matrix turnover and mineralization processes in later stages [276]. Collectively, these findings demonstrate that Cdc42 has multifaceted roles at various stages of amelogenesis, coordinating cytoskeletal organization, metabolic function, and matrix processing to ensure normal enamel development and quality.

4.3.4. Rho GTPases in Dental Roots Development

Dental root development is a tightly regulated morphogenetic process that follows crown formation and involves intricate interactions between epithelial and mesenchymal cells. The root is formed through the proliferation and guidance of Hertwig's epithelial root sheath (HERS), the differentiation of dental papilla mesenchymal cells into odontoblasts, and the organization of dental follicle cells into cementoblasts and periodontal ligament fibroblasts. RhoA, Rac1, and Cdc42 orchestrate the cytoskeletal dynamics, cell polarity, migration, and differentiation required for proper morphogenesis of the root–periodontium complex. These Rho GTPases play essential roles in both the epithelial component HERS and the mesenchymal components, including dental papilla and dental follicle cells.

RhoA is critical for maintaining the structural integrity and apical-basal polarity of HERS cells, which guide root elongation and patterning. Through its downstream effector ROCK, RhoA organizes actin filaments and cell–cell junctions, enabling HERS extension and signaling to the underlying mesenchyme [277]. In dental papilla cells, RhoA regulates odontoblast differentiation by orchestrating cytoskeletal remodeling and establishing cell elongation and polarity, which are prerequisites for directional secretion of dentin matrix components [241,244]. RhoA also influences transcriptional regulation via YAP/TAZ mechanotransduction pathways, allowing cells to respond to ECM stiffness and tissue-level forces [61,253]. Furthermore, RhoA interacts with TGF-β and non-canonical Wnt signaling to fine-tune differentiation cues in the root-forming niche [11,230,239,242,244,252]. Genetic disruption of upstream regulators such as Trio (a Rho-GEF) or Din results in defective root formation [278,279], further validating the central role of RhoA-GTPase signaling in coordinating epithelial-mesenchymal interactions and cytoskeletal dynamics during root development.

Although direct evidence for Rac1's role in root development is limited, deletion of Trio—the upstream regulator of RhoA and Rac1—disrupts root formation, implicating downstream Rho GTPases in this process [279]. Given Rac1's well-established roles in cytoskeletal regulation, cell migration, and adhesion, it is likely to contribute to root morphogenesis. Rac1 promotes lamellipodia formation and integrin-mediated adhesion, supports the migration and proliferation of mesenchymal progenitors, and facilitates cell–matrix interactions essential for root development [9,26,90,280]. These findings position Rac1 as a probable, though not yet fully defined, regulator of root-supporting structures through its effects on cell behavior and differentiation.

Compared to Rac1, there is more direct evidence supporting the involvement of Cdc42 in root development. Cdc42 plays a functional role in tooth root development by regulating odontogenic differentiation and proliferation of dental pulp cells. Specifically, **Cdc42 acts downstream of Wnt signaling** to enhance the expression of root-related genes and differentiation markers. Canonical Wnt activation promotes nuclear β-catenin accumulation, where Cdc42 contributes to transcriptional responses that drive odontoblast maturation [242]. Additionally, **non-canonical Wnt signaling through Ror2 regulates Cdc42 activity** to control DPC proliferation. In this pathway, Wnt5a or related ligands engage Ror2 receptors, triggering intracellular activation of Cdc42 and subsequent modulation of the cytoskeleton and cell cycle. This coordination ensures sufficient progenitor expansion during the elongation phase of root development [262]. Although its role in HERS remains uncharacterized, current evidence supports Cdc42 as a key regulator for proper root formation.

5. Conclusion and Future Directions

 doi:10.20944/preprints202604.1426.v1

Rho GTPases—particularly RhoA, Rac1, and Cdc42—emerge as master regulators of bone and tooth development by integrating extracellular signals into cytoskeletal remodeling, cell polarity, mechanotransduction, vesicular trafficking, and gene expression. Their dynamic activity governs a wide range of cellular processes essential for the differentiation, function, and spatial organization of osteoblasts, osteoclasts, ameloblasts, odontoblasts, and stem/progenitor cells across skeletal and dental tissues.

In bone, RhoA signaling promotes osteoblast proliferation and mineralization through the ROCK and mDia pathways, and modulates osteoclast function via NF-κB and integrin signaling. Cdc42 orchestrates osteoblast polarity and maturation through actin remodeling and regulates osteoclast sealing zone formation. Rac1 enhances osteogenic differentiation and matrix production by facilitating actin polymerization and Wnt/β-catenin signaling. These GTPases also play indispensable roles SSC self-renewal, lineage commitment, and response to mechanical cues, largely mediated by pathways such as YAP/TAZ, BMP, TGF-β, and FGF.

In the context of dental development, Rho GTPases govern epithelial-mesenchymal interactions and morphogenesis at multiple stages. RhoA-ROCK signaling is essential for epithelial bending, odontoblast polarization, and dentin matrix secretion. Rac1 modulates cell migration, polarity, and lamellipodia formation, supporting the organization of both ameloblasts and odontoblasts. While Rac1's role in tooth root development remains indirect, Cdc42 has more definitive evidence of involvement. It regulates root elongation and odontogenic differentiation by integrating canonical Wnt activation and non-canonical Wnt5a–Ror2 signaling. Cdc42 also controls apical-basal polarity via PAR3–PAR6–aPKC complexes and participates in JNK-mediated cytoskeletal remodeling, crucial for dentinal tubule patterning. Furthermore, recent data show that disruption of upstream regulators such as Trio or Din, which modulate RhoA/Cdc42 activity, leads to defective root formation, further reinforcing their importance in root morphogenesis.

Despite these advances, key questions remain. First, the **isoform-specific functions** and **context-dependent effects** of RhoA, Rac1, and Cdc42 in distinct skeletal and dental compartments are still incompletely understood. Second, the **crosstalk between Rho GTPases and major developmental signaling pathways** (Wnt, BMP, TGF-β, FGF, Notch, Hippo-YAP/TAZ) requires deeper mechanistic dissection. Third, the **spatiotemporal dynamics of Rho GTPase activity** during in vivo development, injury response, and regeneration remain difficult to capture with current genetic and imaging tools.

Technological advances will be critical for addressing these gaps. Future studies should utilize:

- **Inducible and lineage-specific Cre models** to dissect stage- and cell-type-specific functions.
- **Live-cell imaging and biosensors** to track Rho GTPase activity in real time.
- **Single-cell transcriptomics and proteomics** to uncover cell-state-specific Rho signaling networks.
- **3D organoid and explant models**, particularly in dental research, to better model tissue complexity.
- **Biomechanically tunable materials** to study how Rho GTPases interpret physical cues in development and repair.

Therapeutically, targeting Rho GTPase pathways offers promising avenues for **bone regeneration, periodontal repair**, and **treatment of craniofacial anomalies**. Pharmacologic inhibitors of RhoA effectors (e.g., ROCK) are already under investigation for their potential in enhancing osteogenesis or limiting pathological bone resorption. However, the **challenge of achieving spatiotemporal specificity and minimizing systemic toxicity** remains. Therefore, strategies such as localized drug delivery, biomimetic scaffolds, and gene-editing technologies may be needed to harness the full therapeutic potential of Rho GTPase modulation.

In conclusion, Rho GTPases function as central molecular hubs that integrate mechanical and chemical cues during bone and tooth development. A more refined understanding of their regulatory logic will not only elucidate fundamental mechanisms of craniofacial and skeletal biology but also facilitate the development of targeted therapies for musculoskeletal and dental disorders.

 doi:10.20944/preprints202604.1426.v1

Author Contributions: S. Thapa, B. Lammichane, Y Zhang, and X. Wang contributed to conception, design, data analysis, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

Acknowledgments: The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by NIH grant DE033676 (X. Wang).

Conflicts of Interests: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Acknowledgments of Literature Scope: We sincerely apologize to those researchers whose important contributions could not be included in this review due to space limitations or the specific focus of our discussion. We recognize the depth and breadth of work in this field and encourage readers to consult the broader literature for a more comprehensive understanding.

Table 1. Roles of Rho GTPases in Bone Biology.

Feature / Process	RhoA	Rac1	Cdc42
Actin Cytoskeletal Structures	Stress fibers, focal adhesions via ROCK/mDia-mediated contractility [55,58]	Lamellipodia, membrane ruffles via actin polymerization [80–83]	Filopodia, polarity complexes, Golgi positioning, vesicle trafficking [32,33]
Osteoblast Differentiation	ROCK–YAP/TAZ and MRTF-A mechanotransduction; context-dependent [59–63]	PAK–MAPK/ERK–Runx2 and Wnt/β-catenin pathways [80–88]	Polarity regulation and ERK/Smad, PAK signaling → Runx2 [49,50]
Osteoclast Differentiation	Sealing zone formation via ROCK; NF-κB/NFATc1 regulation [127–129]	Podosome formation; RANKL–NF-κB signaling [124,125]	Regulates proliferation, polarity, MITF/NFATc1 [115,126]
Bone Resorption	Sealing zone, actomyosin contractility (ROCK–MLC axis) [127,135]	Ruffled border formation, vesicle trafficking, enzyme secretion [122,164]	Vesicle delivery and cytoskeletal coordination [121,182]
Migration (SSCs and Osteoclasts)	Contractility and retraction via ROCK-mediated tension [55,58]	Lamellipodia-driven migration and spreading [90,91]	Directional migration via Par3/Par6/aPKC complex [115]
ECM Interaction & Mechanotransduction	ECM–integrin–FAK–Src–GEF–RhoA axis; YAP/TAZ and MRTF-A activation [40,61,64]	Integrin-mediated actin remodeling; PAK/MAPK and Wnt/β-catenin signaling [87,106]	ECM-regulated polarity; PAK–LIMK1 and ERK/Smad pathways [39,52]
SSC Proliferation & Expansion	Adhesion-dependent signaling and cytoskeletal tension [55,58]	PAK–PI3K/AKT and growth factor signaling (PDGF, IGF-1, FGF2) [95–100]	Cell cycle progression and spindle orientation [51]
SSC Osteogenic Fate	Rheostat control of osteogenesis [55–57]	Runx2 and β-catenin activation [86,88]	Polarity and niche interaction-dependent osteogenesis [39,51]
Pathological Role	Hyperactivation suppresses Wnt/β-catenin; impairs osteogenesis [44,45]	Dysregulation leads to fragility or pathological ossification [48,102]	Dysregulation contributes to aging and osteoarthritis [52,119]

 doi:10.20944/preprints202604.1426.v1

Therapeutic Implication	Requires precise modulation (not simple inhibition/activation) [40,61]	Context-dependent targeting [101,107]	Requires cell-specific targeting [134,184]

Table 2. Roles of Rho GTPases in Odontogenesis.

Feature / Process	RhoA	Rac1	Cdc42
Early Tooth Morphogenesis	Epithelial bending, invagination, basal constriction via ROCK-mediated actomyosin; disruption impairs bud formation [218–222]	Actin polymerization, epithelial rearrangement, invagination; maintains epithelial integrity [216,235]	Epithelial organization and polarity in enamel organ [213]
Epithelial–Mesenchymal Interaction & Mesenchymal Behavior	Mesenchymal migration, adhesion, condensation via integrin and stress fibers; stabilizes epithelial–mesenchymal interface [223–225]	Coordinates epithelial migration, cytoskeletal regulation, integrin-mediated adhesion [236,237]	Maintains polarity-dependent epithelial organization for signaling [213]
Signaling Integration (Early Stage)	Interacts with Wnt/β-catenin, BMP, FGF; regulates YAP/TAZ via cytoskeletal tension [11,61,230]	Interacts with Wnt/β-catenin and integrin signaling [86,237]	Primarily regulates polarity; limited direct signaling described [213]
Dentinogenesis – Odontoblast Differentiation	Odontoblast elongation, alignment, polarization via ROCK-mediated cytoskeletal remodeling [239,240]	Odontoblast polarity, alignment, morphology via actin dynamics [214,239]	Apical–basal polarity and cytoskeletal organization [239,241]
Dentinogenesis – Matrix Secretion	Polarized secretion of collagen, DSPP, DMP1 via cytoskeletal/microtubule coordination [241,244]	Vesicular transport and secretion of dentin matrix proteins; disruption causes defects [214,258]	Golgi positioning, vesicle trafficking, directional secretion [241]
Dentinogenesis – Signaling Pathways	YAP/TAZ; BMP, TGF-β, Wnt; enhances SMAD signaling [239,244,251–253]	Wnt/β-catenin, MAPK, TGF-β signaling [86,260]	Wnt5a–Ror2–Cdc42–JNK; PAR polarity complex [260,261]
Amelogenesis – Early Stage	Ameloblast elongation, cytoskeleton, polarity via ROCK [17,263,265]	Epithelial polarity, spreading, laminin–integrin interactions [17,275]	Polarity, cytoskeleton, cell–cell adhesion; enamel organ integrity [213]
Amelogenesis – Secretory Stage	Tomes' process integrity; actin organization, vesicle trafficking; junction maintenance [270,271]	Required for enamel matrix secretion; loss causes enamel defects [216]	Planar polarity, junction integrity, synchronized secretion [264]
Amelogenesis – Maturation Stage	Cytoskeletal remodeling, actomyosin contractility during mineralization [273]	Not specifically detailed	Enamel protein resorption, mineralization, metabolism [276]

 doi:10.20944/preprints202604.1426.v1

Root Development	HERS polarity, cytoskeleton via ROCK; odontoblast differentiation; YAP/TAZ; TGF-β/Wnt [241,253]	Indirect via Trio; migration, adhesion, proliferation [279]	Dental pulp proliferation and differentiation via Wnt/β-catenin and Ror2 [262]
Functional Consequences of Dysregulation	Impaired invagination, defective dentin, disrupted polarity, enamel abnormalities [240,241]	Defective epithelial organization, impaired dentinogenesis, enamel defects [214,216]	Loss of polarity, enamel defects, oxidative stress, abnormal root development [18,213]

References

1. Liu J., Gao J., Liang Z., Gao C., Niu Q., Wu F., Zhang L. Mesenchymal stem cells and their microenvironment. *Stem Cell Res. Ther.* ***2022***, 13, 429.
2. Boyle W.J., Simonet W.S., Lacey D.L. Osteoclast differentiation and activation. *Nature* ***2003***, 423, 337-342.
3. Thesleff, I. **Epithelial–mesenchymal signalling regulating tooth morphogenesis.** *J. Cell Sci. 2003, 116*, 1647-1648.
4. Yu T., Klein O.D. Molecular and cellular mechanisms of tooth development, homeostasis and repair. *Development* ***2020***, 147, dev184754.
5. Jaffe A.B., Hall A. Rho GTPases: biochemistry and biology. *Annu. Rev. Cell Dev. Biol.* ***2005***, 21, 247-269.
6. Bos J.L., Rehmann H., Wittinghofer A. GEFs and GAPs: critical elements in the control of small G proteins. *Cell* ***2007***, 129, 865-877.
7. Heasman S.J., Ridley A.J. Mammalian Rho GTPases: new insights into their functions from in vivo studies. *Nat. Rev. Mol. Cell Biol.* ***2008***, 9, 690-701.
8. Chandrasekar S., Beach J.R., Oakes P.W. Shining a light on RhoA: Optical control of cell contractility. *Int. J. Biochem. Cell Biol.* ***2023***, 161,106442.
9. Mehidi A., Rossier O., Schaks M., Chazeau A., Binamé F., Remorino A., Coppey M., Karatas Z., Sibarita J.B., Rottner K., Moreau V., Giannone G. Transient Activations of Rac1 at the Lamellipodium Tip Trigger Membrane Protrusion. *Curr. Biol.* ***2019***, 29, 2852-2866.e5.
10. Pichaud F., Walther R.F., Nunes de Almeida F. Regulation of Cdc42 and its effectors in epithelial morphogenesis. *J. Cell Sci.* ***2019***, 132, jcs217869.
11. Schlessinger K., Hall A., Tolwinski N. Wnt signaling pathways meet Rho GTPases. *Genes Dev.* ***2009***, 23, 265-277.
12. Leask A., Abraham D.J. TGF-beta signaling and the fibrotic response. *FASEB J.* ***2004***, 18, 816-827.
13. Li N., Lu N., Xie C. The Hippo and Wnt signalling pathways: crosstalk during neoplastic progression in gastrointestinal tissue. *FEBS J.* ***2019***, 286, 3745-3756.
14. Wei Y., Hui V.L.Z., Chen Y., Han R., Han X., Guo Y. YAP/TAZ: Molecular pathway and disease therapy. *MedComm. (2020)* ***2023***, 4, e340.
15. Zeng R., Zhuo Z/, Luo Y/, Sha W/, Chen H. Rho GTPase signaling in rheumatic diseases. *iScience* ***2021***, 25, 103620.
16. Deng Z., Jia Y., Liu H., He M., Yang Y., Xiao W., Li Y. RhoA/ROCK pathway: implication in osteoarthritis and therapeutic targets. *Am. J. Transl. Res.* ***2019***, 11, 5324-5331.
17. Otsu K., Harada H. Rho GTPases in ameloblast differentiation. *Jpn. Dent. Sci. Rev.* ***2016***, 52, 32-40.
18. Zheng J., Yu R., Tang Y., Su S., Wang S., Liao C., Li X., Liao J., Yu D., Ai T., Zhao W., Yau V., Liu C., Wu L., Cao Y. *Cdc42* deletion yielded enamel defects by disrupting mitochondria and producing reactive oxygen species in dental epithelium. *Genes Dis.* ***2023***, 11, 101194.
19. Wennerberg K., Rossman K.L., Der C.J. The Ras superfamily at a glance. *J. Cell Sci.* ***2005***, 118, 843-846.
20. Cherfils J., Zeghouf M. Regulation of small GTPases by GEFs, GAPs, and GDIs. *Physiol. Rev.* ***2013***, 93, 269-309.

 doi:10.20944/preprints202604.1426.v1

21. Zaidel-Bar R., Zhenhuan G., Luxenburg C. The contractome--a systems view of actomyosin contractility in non-muscle cells. *J. Cell Sci.* ***2015***,128, 2209-2217.
22. Acharya B.R., Nestor-Bergmann A., Liang X., Gupta S., Duszyc K., Gauquelin E., Gomez G.A., Budnar S., Marcq P., Jensen O.E., Bryant Z., Yap A.S. A Mechanosensitive RhoA Pathway that Protects Epithelia against Acute Tensile Stress. *Dev. Cell* ***2018***, 47, 439-452.e6.
23. Kassianidou E., Hughes J.H., Kumar S. Activation of ROCK and MLCK tunes regional stress fiber formation and mechanics via preferential myosin light chain phosphorylation. *Mol. Biol. Cell* ***2017***, 28, 3832-3843.
24. Olson E.N., Nordheim A. Linking actin dynamics and gene transcription to drive cellular motile functions. Nat. Rev. Mol. *Cell Biol.* ***2010***, 11, 353-365.
25. Ding B., Yang S., Schaks M., Liu Y., Brown A.J., Rottner K., Chowdhury S., Chen B. Structures reveal a key mechanism of WAVE regulatory complex activation by Rac1 GTPase. *Nat. Commun.* ***2022***, 13, 5444.
26. Steffen A., Ladwein M., Dimchev G.A., Hein A., Schwenkmezger L., Arens S., Ladwein K.I., Margit Holleboom J., Schur F., Victor Small J., Schwarz J., Gerhard R., Faix J., Stradal T.E., Brakebusch C., Rottner K. Rac function is crucial for cell migration but is not required for spreading and focal adhesion formation. *J. Cell Sci.* ***2013***, 126, 4572-88.
27. Gastonguay A., Berg T., Hauser A.D., Schuld N., Lorimer E., Williams C.L. The role of Rac1 in the regulation of NF-κB activity, cell proliferation, and cell migration in non-small cell lung carcinoma. *Cancer Biol. Ther.* ***2012***, 13, 647-656.
28. Myant K.B., Cammareri P., McGhee E.J., Ridgway R.A., Huels D.J., Cordero J.B., Schwitalla S., Kalna G., Ogg E.L., Athineos D., Timpson P., Vidal M., Murray G.I., Greten F.R., Anderson K.I., Sansom O.J. ROS production and NF-κB activation triggered by RAC1 facilitate WNT-driven intestinal stem cell proliferation and colorectal cancer initiation. *Cell Stem Cell* ***2013***, 12, 761-773.
29. Chen J., Li Q., Hong Y., Zhou X., Yu C., Tian X., Zhao J., Long C., Shen L., Wu S., Wei G. Inhibition of the NF-κB Signaling Pathway Alleviates Pyroptosis in Bladder Epithelial Cells and Neurogenic Bladder Fibrosis. *Int. J. Mol. Sci.* ***2023***, 24, 11160.
30. Campellone K.G., Lebek N.M., King V.L. Branching out in different directions: Emerging cellular functions for the Arp2/3 complex and WASP-family actin nucleation factors. *Eur. J. Cell Biol.* ***2023***, 102, 151301.
31. Mascanzoni F., Iannitti R., Colanzi A. Functional Coordination among the Golgi Complex, the Centrosome and the Microtubule Cytoskeleton during the Cell Cycle. *Cells* ***2022***, 11, 354.
32. Harris K.P., Tepass U. Cdc42 and vesicle trafficking in polarized cells. *Traffic* ***2010***, 11, 1272-1279.
33. Hehnly H., Xu W., Chen J.L., Stamnes M. Cdc42 regulates microtubule-dependent Golgi positioning. *Traffic* ***2010***, 11, 1067-1078.
34. Machacek M., Hodgson L., Welch C., Elliott H., Pertz O., Nalbant P., Abell A., Johnson G.L., Hahn K.M., Danuser G. Coordination of Rho GTPase activities during cell protrusion. *Nature* ***2009***, 461, 99-103.
35. Zegers M.M., Friedl P. Rho GTPases in collective cell migration. *Small GTPases* ***2014***, 5:e28997.
36. Martin K., Reimann A., Fritz R.D., Ryu H., Jeon N.L., Pertz O. Spatio-temporal co-ordination of RhoA, Rac1 and Cdc42 activation during prototypical edge protrusion and retraction dynamics. *Sci. Rep.* ***2016***, 6, 21901.
37. Wan Q., Cho E., Yokota H., Na S. Rac1 and Cdc42 GTPases regulate shear stress-driven β-catenin signaling in osteoblasts. *Biochem. Biophys. Res. Commun.* ***2013***, 433, 502-507.
38. Huck K., Sens C., Wuerfel C., Zoeller C., Nakchbandi I.A. The Rho GTPase RAC1 in Osteoblasts Controls Their Function. *Int. J. Mol. Sci.* ***2020***, 21, 385. Rac1 in preosteoblasts is required for normal osteoblast function and bone density, but it is neither needed for PTH-mediated anabolic effects nor for integrin-mediated enhancement of differentiation.
39. Meyers V.E., Zayzafoon M., Douglas J.T., McDonald J.M. RhoA and cytoskeletal disruption mediate reduced osteoblastogenesis and enhanced adipogenesis of human mesenchymal stem cells in modeled microgravity. *J. Bone Miner. Res.* ***2005***, 20, 1858-1866.
40. Burridge K., Monaghan-Benson E., Graham D.M. Mechanotransduction: from the cell surface to the nucleus via RhoA. Philos. *Trans. R. Soc. Lond. B. Biol. Sci.* ***2019***, 374, 20180229.
41. Dang I., Brazzo J.A., Bae Y., Assoian R.K. Key role for Rac in the early transcriptional response to extracellular matrix stiffness and stiffness-dependent repression of ATF3. *J. Cell Sci.* ***2023***, 136, jcs260636.

 doi:10.20944/preprints202604.1426.v1

42. Melendez J., Grogg M., Zheng Y. Signaling role of Cdc42 in regulating mammalian physiology. *J. Biol. Chem.* ***2011***, 286, 2375-2381.
43. Strzelecka-Kiliszek A., Mebarek S., Roszkowska M., Buchet R., Magne D., Pikula S. Functions of Rho family of small GTPases and Rho-associated coiled-coil kinases in bone cells during differentiation and mineralization. *Biochim. Biophys. Acta. Gen. Subj.* ***2017***, 1861, 1009-1023.
44. Ge J., Burnier L., Adamopoulou M., Kwa M.Q., Schaks M., Rottner K., Brakebusch C. RhoA, Rac1, and Cdc42 differentially regulate αSMA and collagen I expression in mesenchymal stem cells. *J. Biol. Chem.* ***2018***, 293, 9358-9369.
45. Wang J., Xu C., Zhang J., Bao Y., Tang Y., Lv X., Ma B., Wu X., Mao G. RhoA promotes osteoclastogenesis and regulates bone remodeling through mTOR-NFATc1 signaling. *Mol. Med.* ***2023***, 29, 49.
46. Khatiwala C.B., Kim P.D., Peyton S.R., Putnam A.J. ECM compliance regulates osteogenesis by influencing MAPK signaling downstream of RhoA and ROCK. *J. Bone Miner. Res.* ***2009***, *24*, 886–898.
47. Hamamura K., Swarnkar G., Tanjung N., Cho E., Li J., Na S., Yokota H. RhoA-mediated signaling in mechanotransduction of osteoblasts. *Connect. Tissue Res.* ***2012***, *53*, 398–406.
48. Blangy A., Bompard G., Guerit D., Marie P., Maurin J., Morel A., Vives V. The osteoclast cytoskeleton—current understanding and therapeutic perspectives for osteoporosis. *J. Cell Sci.* ***2020***, *133*, jcs244798.
49. Heasman S.J., Ridley A.J. Mammalian Rho GTPases: new insights into their functions from in vivo studies. *Nat. Rev. Mol. Cell Biol.* ***2008***, 9, 690-701.
50. Ye J., Wang J., Zhao J., Xia M., Wang H., Sun L., Zhang W.B. RhoA/ROCK-TAZ Axis regulates bone formation within calvarial trans-sutural distraction osteogenesis. *Cell Signal.* ***2024***, *121*, 111300.
51. Khatiwala C.B., Kim P.D., Peyton S.R., Putnam A.J. ECM compliance regulates osteogenesis by influencing MAPK signaling downstream of RhoA and ROCK. *J. Bone Miner. Res.* ***2009***, *24*, 886–898.
52. Narumiya S., Thumkeo D. Rho signaling research: history, current status and future directions. *FEBS Lett.* ***2018***, *592*, 1763–1776.
53. Arnsdorf E.J., Tummala P., Kwon R.Y., Jacobs C.R. Mechanically induced osteogenic differentiation—the role of RhoA, ROCKII and cytoskeletal dynamics. *J. Cell Sci.* ***2009***, *122*, 546–553.
54. Wei L., Zhou W., Croissant J.D., Johansen F.E., Prywes R., Balasubramanyam A., Schwartz R.J. RhoA signaling via serum response factor plays an obligatory role in myogenic differentiation. *J. Biol. Chem.* ***1998***, *273*, 30287–30294.
55. Bian H., Lin J.Z., Li C., Farmer S.R. Myocardin-related transcription factor A (MRTFA) regulates the fate of bone marrow mesenchymal stem cells and its absence in mice leads to osteopenia. *Mol. Metab.* ***2016***, *5*, 970–979.
56. Dai J., Qin L., Chen Y., Wang H., Lin G., Li X., Liao H., Fang H. Matrix stiffness regulates epithelial-mesenchymal transition via cytoskeletal remodeling and MRTF-A translocation in osteosarcoma cells. *J. Mech. Behav. Biomed. Mater.* ***2019***, *90*, 226–238.
57. 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*, 14326.
58. Hong S.Y., Jeon Y.M., Lee H.J., Kim J.G., Baek J.A., Lee J.C. Activation of RhoA and FAK induces ERK-mediated osteopontin expression in mechanical force-subjected periodontal ligament fibroblasts. *Mol. Cell. Biochem.* ***2010***, *335*, 263–272.
59. McBeath R., Pirone D.M., Nelson C.M., Bhadriraju K., Chen C.S. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev. Cell* ***2004***, *6*, 483–495.
60. Shih Y.R., Tseng K.F., Lai H.Y., Lin C.H., Lee O.K. Matrix stiffness regulation of integrin-mediated mechanotransduction during osteogenic differentiation of human mesenchymal stem cells. *J. Bone Miner. Res.* ***2011***, *26*, 730–738.
61. Zhang Q., Lin S., Zhang T., Tian T., Ma Q., Xie X., Xue C., Lin Y., Zhu B., Cai X. Curved microstructures promote osteogenesis of mesenchymal stem cells via the RhoA/ROCK pathway. *Cell Prolif.* ***2017***, *50*, e12356.
62. Huang X., Chen X., Chen H., Xu D., Lin C., Peng B. Rho/Rho-associated protein kinase signaling pathway-mediated downregulation of runt-related transcription factor 2 expression promotes the differentiation of dental pulp stem cells into odontoblasts. *Exp. Ther. Med.* ***2018***, *15*, 4457–4464.

 doi:10.20944/preprints202604.1426.v1

63. Shi W., Xu C., Gong Y., Wang J., Ren Q., Yan Z., Mei L., Tang C., Ji X., Hu X., Qv M., Hussain M., Zeng L.H., Wu X. RhoA/ROCK activation represents a new mechanism for inactivating Wnt/β-catenin signaling in the aging-associated bone loss. *Cell Regen.* ***2021***, *10*, 8.
64. Lane S.W., De Vita S., Alexander K.A., Karaman R., Milsom M.D., Dorrance A.M., Purdon A., Louis L., Bouxsein M.L., Williams D.A. Rac signaling in osteoblastic cells is required for normal bone development but is dispensable for hematopoietic development. *Blood* ***2012***, *119*, 736–744.
65. Sugihara K., Nakatsuji N., Nakamura K., Nakao K., Hashimoto R., Otani H., Sakagami H., Kondo H., Nozawa S., Aiba A., Katsuki M. Rac1 is required for the formation of three germ layers during gastrulation. *Oncogene* ***1998***, *17*, 3427–3433.
66. Onishi M., Fujita Y., Yoshikawa H., Yamashita T. Inhibition of Rac1 promotes BMP-2-induced osteoblastic differentiation. *Cell Death Dis.* ***2013***, *4*, e698.
67. Suzuki D., Yamada A., Amano T., Yasuhara R., Kimura A., Sakahara M., Tsumaki N., Takeda S., Tamura M., Nakamura M., Wada N., Nohno T., Shiroishi T., Aiba A., Kamijo R. Essential mesenchymal role of small GTPase Rac1 in interdigital programmed cell death during limb development. *Dev. Biol.* ***2009***, *335*, 396–406.
68. Wang G., Woods A., Agoston H., Ulici V., Glogauer M., Beier F. Genetic ablation of Rac1 in cartilage results in chondrodysplasia. *Dev. Biol.* ***2007***, *306*, 612–623.
69. Suzuki D., Bush J.R., Bryce D.M., Kamijo R., Beier F. Rac1 dosage is crucial for normal endochondral bone growth. *Endocrinology* ***2017***, *158*, 3386–3398.
70. Prowse P.D., Elliott C.G., Hutter J., Hamilton D.W. Inhibition of Rac and ROCK signalling influence osteoblast adhesion, differentiation and mineralization on titanium topographies. *PLoS One* ***2013***, *8*, e58898.
71. Ge C., Xiao G., Jiang D., Yang Q., Hatch N.E., Roca H., Franceschi R.T. Identification and functional characterization of ERK/MAPK phosphorylation sites in the Runx2 transcription factor. *J. Biol. Chem.* ***2009***, *284*, 32533–32543.
72. Li Y., Ge C., Franceschi R.T. MAP kinase-dependent RUNX2 phosphorylation is necessary for epigenetic modification of chromatin during osteoblast differentiation. *J. Cell. Physiol.* ***2017***, *232*, 2427–2435.
73. Jung G.Y., Park Y.J., Han J.S. Mediation of Rac1 activation by kindlin-2: an essential function in osteoblast adhesion, spreading, and proliferation. *J. Cell. Biochem.* ***2011***, *112*, 2541–2548.
74. Hendesi H., Barbe M.F., Safadi F.F., Monroy M.A., Popoff S.N. Integrin-mediated adhesion of osteoblasts to connective tissue growth factor (CTGF/CCN2) induces cytoskeleton reorganization and cell differentiation. *PLoS One* ***2015***, *10*, e0115325.
75. Zhang X., Liang X., Wen Y., Wu F., Gao G., Zhang L., Gu Y., Zhang J., Zhou F., Li W., Tang L., Yang X., Zhao H., Zhou C., Hirsch F.R. RAC1 inhibition ameliorates IBSP-induced bone metastasis in lung adenocarcinoma. *Cell Rep.* ***2024***, *43*, 114528.
76. Wang X., Tang P., Guo F., Zhang M., Yan Y., Huang M., Chen Y., Zhang L., Zhang L. mDia1 and Cdc42 regulate Activin B-induced migration of bone marrow-derived mesenchymal stromal cells. *Stem Cells* ***2019***, *37*, 150–162.
77. Wirth F., Huck K., Lubosch A., Zoeller C., Ghura H., Porubsky S., Nakchbandi I.A. Cdc42 in osterix-expressing cells alters osteoblast behavior and myeloid lineage commitment. *Bone* ***2021***, *153*, 116150.
78. Yang L., Wang L., Zheng Y. Gene targeting of Cdc42 and Cdc42GAP affirms the critical involvement of Cdc42 in filopodia induction, directed migration, and proliferation in primary mouse embryonic fibroblasts. *Mol. Biol. Cell* ***2006***, *17*, 4675–4685.
79. Bagrodia S., Dérijard B., Davis R.J., Cerione R.A. Cdc42 and PAK-mediated signaling leads to Jun kinase and p38 mitogen-activated protein kinase activation. *J. Biol. Chem.* ***1995***, *270*, 27995–27998.
80. Gorski J.L., Estrada L., Hu C., Liu Z. Skeletal-specific expression of Fgd1 during bone formation and skeletal defects in faciogenital dysplasia (FGDY; Aarskog syndrome). *Dev. Dyn.* ***2000***, *218*, 573–586.
81. Zou W., Greenblatt M.B., Shim J.H., Kant S., Zhai B., Lotinun S., Brady N., Hu D.Z., Gygi S.P., Baron R., Davis R.J., Jones D., Glimcher L.H. MLK3 regulates bone development downstream of the faciogenital dysplasia protein FGD1 in mice. *J. Clin. Invest.* ***2011***, *121*, 4383–4392. *FGD1* encodes a guanine nucleotide exchange factor (GEF) that specifically activates the Rho GTPase CDC42. MLK3 functions downstream of FGD1 to regulate ERK and p38 MAPK, which in turn phosphorylate and activate the master regulator of osteoblast differentiation, Runx2.

 doi:10.20944/preprints202604.1426.v1

82. Gao L., Gorski J.L., Chen C.S. The Cdc42 guanine nucleotide exchange factor FGD1 regulates osteogenesis in human mesenchymal stem cells. *Am. J. Pathol.* ***2011***, *178*, 969–974.
83. Kho M., Hladyshau S., Tsygankov D., Nie S. Coordinated regulation of Cdc42ep1, actin, and septin filaments during neural crest cell migration. *Front. Cell Dev. Biol.* ***2023***, *11*, 1106595.
84. Qiao M., Shapiro P., Fosbrink M., Rus H., Kumar R., Passaniti A. Cell cycle-dependent phosphorylation of the RUNX2 transcription factor by cdc2 regulates endothelial cell proliferation. *J. Biol. Chem.* ***2006***, *281*, 7118–7128.
85. Li Z., Wu J., Zhang X., Ou C., Zhong X., Chen Y., Lu L., Liu H., Li Y., Liu X., Wu B., Wang Y., Yang P., Yan J., Chen M. CDC42 promotes vascular calcification in chronic kidney disease. *J. Pathol.* ***2019***, *249*, 461–471.
86. Weivoda M.M., Oursler M.J. The roles of small GTPases in osteoclast biology. *Orthop. Muscular Syst.* ***2014***, *3*, 1000161.
87. Xu S., Zhang Y., Wang J., Li K., Tan K., Liang K., Shen J., Cai D., Jin D., Li M., Xiao G., Xu J., Jiang Y., Bai X. TSC1 regulates osteoclast podosome organization and bone resorption through mTORC1 and Rac1/Cdc42. *Cell Death Differ.* ***2018***, *25*, 1549–1566.
88. Liu Y., Dou Y., Yan L., Yang X., He B., Kong L., Smith W. The role of Rho GTPases' substrates Rac and Cdc42 in osteoclastogenesis and relevant natural medicinal products study. *Biosci. Rep.* ***2020***, *40*, BSR20200407.
89. Lee N.K., Choi Y.G., Baik J.Y., Han S.Y., Jeong D.W., Bae Y.S., Kim N., Lee S.Y. A crucial role for reactive oxygen species in RANKL-induced osteoclast differentiation. *Blood* ***2005***, *106*, 852–859.
90. Kim J.H., Kim N. Signaling pathways in osteoclast differentiation. *Chonnam Med. J.* ***2016***, *52*, 12–17.
91. Teenblock C., Heckel T., Czupalla C., Espírito Santo A.I., Niehage C., Sztacho M., Hoflack B. The Cdc42 guanine nucleotide exchange factor FGD6 coordinates cell polarity and endosomal membrane recycling in osteoclasts. *J. Biol. Chem.* ***2014***, *289*, 18347–18359.
92. Gu J., Yang Z., Yuan L., Guo S., Wang D., Zhao N., Meng L., Liu H., Chen W., Ma J. Rho-GEF trio regulates osteoclast differentiation and function by Rac1/Cdc42. *Exp. Cell Res.* ***2020***, *396*, 112265.
93. Touaitahuata H., Blangy A., Vives V. Modulation of osteoclast differentiation and bone resorption by Rho GTPases. *Small GTPases* ***2014***, *5*, e28119.
94. Morel A., Douat C., Blangy A., Vives V. Bone resorption by osteoclasts involves fine tuning of RHOA activity by its microtubule-associated exchange factor GEF-H1. *Front. Physiol.* ***2024***, *15*, 1342024.
95. Teitelbaum S.L. Bone resorption by osteoclasts. *Science* ***2000***, *289*, 1504–1508.
96. Chellaiah M.A. Regulation of actin ring formation by Rho GTPases in osteoclasts. *J. Biol. Chem.* ***2005***, *280*, 32930–32943.
97. Ory S., Brazier H., Pawlak G., Blangy A. Rho GTPases in osteoclasts: orchestrators of podosome arrangement. *Eur. J. Cell Biol.* ***2008***, *87*, 469–477.
98. McMichael B.K., Cheney R.E., Lee B.S. Myosin X regulates sealing zone patterning in osteoclasts through linkage of podosomes and microtubules. *J. Biol. Chem.* ***2010***, *285*, 9506–9515.
99. Totsukawa G., Yamakita Y., Yamashiro S., Hartshorne D.J., Sasaki Y., Matsumura F. Distinct roles of ROCK (Rho-kinase) and MLCK in spatial regulation of MLC phosphorylation for assembly of stress fibers and focal adhesions in 3T3 fibroblasts. *J. Cell Biol.* ***2000***, *150*, 797–806.
100. Deng Z., Jia Y., Liu H., He M., Yang Y., Xiao W., Li Y. RhoA/ROCK pathway: implication in osteoarthritis and therapeutic targets. *Am. J. Transl. Res.* ***2019***, *11*, 5324–5331.
101. Chellaiah M.A., Soga N., Swanson S., McAllister S., Alvarez U., Wang D., Dowdy S.F., Hruska K.A. Rho-A is critical for osteoclast podosome organization, motility, and bone resorption. *J. Biol. Chem.* ***2000***, *275*, 11993–12002.
102. Rodríguez-Trillo A., Pena C., García S., Pérez-Pampín E., Rodríguez-López M., Mera-Varela A., González A., Conde C. ROCK inhibition with Y-27632 reduces joint inflammation and damage in serum-induced arthritis model and decreases in vitro osteoclastogenesis in patients with early arthritis. *Front. Immunol.* ***2022***, *13*, 858069.
103. Takayanagi H., Kim S., Koga T., Nishina H., Isshiki M., Yoshida H., Saiura A., Isobe M., Yokochi T., Inoue J., Wagner E.F., Mak T.W., Kodama T., Taniguchi T. Induction and activation of the transcription factor

 doi:10.20944/preprints202604.1426.v1

NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. *Dev. Cell* ***2002***, *3*, 889–901.

104. Yamashita T., Yao Z., Li F., Zhang Q., Badell I.R., Schwarz E.M., Takeshita S., Wagner E.F., Noda M., Matsuo K., Xing L., Boyce B.F. NF-kappaB p50 and p52 regulate receptor activator of NF-kappaB ligand (RANKL) and tumor necrosis factor-induced osteoclast precursor differentiation by activating c-Fos and NFATc1. *J. Biol. Chem.* ***2007***, *282*, 18245–18253.
105. Anwar K.N., Fazal F., Malik A.B., Rahman A. RhoA/Rho-associated kinase pathway selectively regulates thrombin-induced intercellular adhesion molecule-1 expression in endothelial cells via activation of IκB kinase β and phosphorylation of RelA/p65. *J. Immunol.* ***2004***, *173*, 6965–6972.
106. Kim H.J., Kim J.G., Moon M.Y., Park S.H., Park J.B. IκB kinase γ/nuclear factor-κB-essential modulator (IKKγ/NEMO) facilitates RhoA GTPase activation, which, in turn, activates Rho-associated kinase (ROCK) to phosphorylate IKKβ in response to transforming growth factor (TGF)-β1. *J. Biol. Chem.* ***2014***, *289*, 1429–1440.
107. Kim J.H., Kim N. Regulation of NFATc1 in osteoclast differentiation. *J. Bone Metab.* ***2014***, *21*, 233–241.
108. Fretz J.A., Shevde N.K., Singh S., Darnay B.G., Pike J.W. Receptor activator of nuclear factor-κB ligand-induced nuclear factor of activated T cells (C1) autoregulates its own expression in osteoclasts and mediates the up-regulation of tartrate-resistant acid phosphatase. *Mol. Endocrinol.* ***2008***, *22*, 737–750.
109. Xu W., Lu Q., Qu M., Fan R., Leng S., Wang L., Liu L., Yang F., Zhou X., Huang D., Zhang L. Wnt4 regulates bone metabolism through IKK-NF-κB and ROCK signaling under occlusal traumatic periodontitis. *J. Periodontal Res.* ***2022***, *57*, 461–469.
110. Duong L.T., Rodan G.A. Integrin-mediated signaling in the regulation of osteoclast adhesion and activation. *Front. Biosci.* ***1998***, *3*, d757–d768.
111. Faccio R., Takeshita S., Zallone A., Ross F.P., Teitelbaum S.L. c-Fms and the αvβ3 integrin collaborate during osteoclast differentiation. *J. Clin. Invest.* ***2003***, *111*, 749–758.
112. Yang L., Chen H., Yang C., Hu Z., Jiang Z., Meng S., Liu R., Huang L., Yang K. Research progress on the regulatory mechanism of integrin-mediated mechanical stress in cells involved in bone metabolism. *J. Cell. Mol. Med.* ***2024***, *28*, e18183.
113. Chen N., Diao C.Y., Huang X., Tan W.X., Chen Y.B., Qian X.Y., Gao J., Zhao D.B. RhoA promotes synovial proliferation and bone erosion in rheumatoid arthritis through Wnt/PCP pathway. *Mediators Inflamm.* ***2023***, *2023*, 5057009.
114. Touaitahuata H., Blangy A., Vives V. Modulation of osteoclast differentiation and bone resorption by Rho GTPases. *Small GTPases* ***2014***, *5*, e28119.
115. Kim J.H., Seong S., Kim K., Kim I., Park J.W., Koh J.T., Kim N. Rac1-dependent regulation of osteoclast and osteoblast differentiation by developmentally regulated GTP-binding 2. *Cell Death Discov.* ***2025***, *11*, 48.
116. Omata Y., Tachibana H., Aizaki Y., Mimura T., Sato K. Essentiality of Nfatc1 short isoform in osteoclast differentiation and its self-regulation. *Sci. Rep.* ***2023***, *13*, 18797.
117. Pang M., Rodríguez-Gonzalez M., Hernandez M., Recinos C.C., Seldeen K.L., Troen B.R. AP-1 and Mitf interact with NFATc1 to stimulate cathepsin K promoter activity in osteoclast precursors. *J. Cell Biochem.* ***2019***, *120*, 12382–12392.
118. Yasuda K., Matsubara T., Shirakawa T., Kawamoto T., Kokabu S. Protein phosphatase 1 regulatory subunit 18 suppresses the transcriptional activity of NFATc1 via regulation of c-Fos. *Bone Rep.* ***2021***, *15*, 101114.
119. Kim S.J., Yoon S. Activated Rac1 regulates the degradation of IκBα and the nuclear translocation of STAT3–NFκB complexes in starved cancer cells. *Exp. Mol. Med.* ***2016***, *48*, e231.
120. Lee N.K., Choi H.K., Kim D.K., Lee S.Y. Rac1 GTPase regulates osteoclast differentiation through TRANCE-induced NF-κB activation. *Mol. Cell. Biochem.* ***2006***, *281*, 55–61. Rac1 may represent a key regulator for differentiation of osteoclasts through the activation of NF-κB
121. Sun Y., Büki K.G., Ettala O., Vääräniemi J.P., Väänänen H.K. Possible role of direct Rac1–Rab7 interaction in ruffled border formation of osteoclasts. *J. Biol. Chem.* ***2005***, *280*, 32356–32361.
122. Roy M., Roux S. Rab GTPases in osteoclastic endomembrane systems. *Biomed. Res. Int.* ***2018***, *2018*, 4541538.
123. Croke M., Ross F.P., Korhonen M., Williams D.A., Zou W., Teitelbaum S.L. Rac deletion in osteoclasts causes severe osteopetrosis. *J. Cell Sci.* ***2011***, *124*, 3811–3821. Rac1 and Rac2 are mutually compensatory.

 doi:10.20944/preprints202604.1426.v1

Unlike Cdc42 deficiency, their combined absence does not impact upon differentiation but promotes severe osteopetrosis by dysregulating the osteoclast cytoskeleton

124. Miki H., Takenawa T. Regulation of actin dynamics by WASP family proteins. *J. Biochem.* ***2003***, *134*, 309–313.
125. Werbenko E., de Gorter D.J.J., Kleimann S., Beckmann D., Waltereit-Kracke V., Reinhardt J., Geers F., Paruzel P., Hansen U., Pap T., Stradal T.E.B., Dankbar B. Hem1 is essential for ruffled border formation in osteoclasts and efficient bone resorption. *Sci. Rep.* ***2024***, *14*, 8109.
126. Marei H., Malliri A. Rac1 in human diseases: The therapeutic potential of targeting Rac1 signaling regulatory mechanisms. *Small GTPases* ***2017***, 8, 139–163.
127. Shin B., Kupferman J., Schmidt E., Polleux F., Delany A.M., Lee S.K. Rac1 inhibition via Srgap2 restrains inflammatory osteoclastogenesis and limits the clastokine SLIT3. *J. Bone Miner. Res.* ***2020***, 35, 789–800.
128. Sima C., Gastfreund S., Sun C., Glogauer M. Rac-null leukocytes are associated with increased inflammation-mediated alveolar bone loss. *Am. J. Pathol.* ***2014***, 184, 472–482.
129. Magalhaes J.K., Grynpas M.D., Willett T.L., Glogauer M. Deleting Rac1 improves vertebral bone quality and resistance to fracture in a murine ovariectomy model. *Osteoporos. Int.* ***2011***, 22, 1481–1492.
130. Yan J., Chen S., Zhang Y., Li X., Li Y., Wu X., Yuan J., Robling A.G., Kapur R., Chan R.J., Yang F.C. Rac1 mediates osteoclast gain-of-function induced by Nf1 haploinsufficiency. *Hum. Mol. Genet.* ***2008***, 17, 936–948.
131. Morel A., Blangy A., Vives V. Methods to investigate the role of Rho GTPases in osteoclast function. *Methods Mol. Biol.* ***2018***, 1821, 219–233.
132. Ito Y., Teitelbaum S.L., Zou W., Zheng Y., Johnson J.F., Chappel J., Ross F.P., Zhao H. Cdc42 regulates bone modeling and remodeling by modulating osteoclast polarization. *J. Clin. Invest.* ***2010***, 120, 1981–1993.
133. Jung Y.K., Kwon K.T., Jang J.A., Han M.S., Kim G.W., Han S. Enhanced activation of Rac1/Cdc42 and MITF leads to augmented osteoclastogenesis in autosomal dominant osteopetrosis type II. *JBMR Plus* ***2018***, 3, e10070.
134. Steenblock C., Heckel T., Czupalla C., Espírito Santo A.I., Niehage C., Sztacho M., Hoflack B. The Cdc42 GEF FGD6 coordinates cell polarity and membrane recycling in osteoclasts. *J. Biol. Chem.* ***2014***, 289, 18347–18359.
135. Blangy A., Bompard G., Guerit D., Marie P., Maurin J., Morel A., Vives V. The osteoclast cytoskeleton: current understanding and therapeutic perspectives. *J. Cell Sci.* ***2020***, 133, jcs244798.
136. Lim Lam V.K., Hin Wong J.Y., Chew S.Y., Chan B.P. Rac1 regulates compression-induced actin protrusions of mesenchymal stem cells. *Biomaterials* ***2021***, 274, 120829.
137. Zhao Y.H., Lv X., Liu Y.L., Zhao Y., Li Q., Chen Y.J., Zhang M. Hydrostatic pressure promotes MSC proliferation and differentiation via RhoA and Rac1. *Stem Cell Res.* ***2015***, 14, 283–296.
138. Liu S., Liu Y., Jiang L., Li Z., Lee S., Liu C., Wang J., Zhang J. BMP-2 accelerates MSC migration via CDC42/PAK1/LIMK1 signaling. *Biomater. Sci.* ***2019***, 7, 362–372.
139. Wang X., Tang P., Guo F., Zhang M., Yan Y., Huang M., Chen Y., Zhang L., Zhang L. mDia1 and Cdc42 regulate MSC migration. *Stem Cells* ***2019***, 37, 150–162.
140. Petzold J., Gentleman E. Intrinsic mechanical cues and their impact on stem cells. *Front. Cell Dev. Biol.* ***2021***, 9, 761871.
141. Xu B., Song G., Ju Y., Li X., Song Y., Watanabe S. RhoA/ROCK signaling in mechanotransduction. *J. Cell Physiol.* ***2012***, 227, 2722–2729.
142. Heng B.C., Zhang X., Aubel D., Bai Y., Li X., Wei Y., Fussenegger M., Deng X. Role of YAP/TAZ in lineage determination. *Front. Cell Dev. Biol.* ***2020***, 8, 735.
143. Tong Z., Liu Y., Xia R., Chang Y., Hu Y., Liu P., Zhai Z., Zhang J., Li H. F-actin regulates osteogenic differentiation via YAP/TAZ. *Nanoscale Res. Lett.* ***2020***, 15, 183.
144. Ma J., Fan H., Geng H. YAP and TAZ in tooth development. *Front. Cell Dev. Biol.* ***2024***, 11, 1281250.
145. Lv H., Li L., Sun M., Zhang Y., Chen L., Rong Y., Li Y. Matrix stiffness regulates stem cell differentiation. *Stem Cell Res. Ther.* ***2015***, 6, 103.
146. Hoon J.L., Tan M.H., Koh C.G. Regulation of cellular responses to mechanical cues by Rho GTPases. *Cells* ***2016***, 5, 17.

 doi:10.20944/preprints202604.1426.v1

147. Li C., Zhen G., Chai Y., Xie L., Crane J.L., Farber E., Farber C.R., Luo X., Gao P., Cao X., Wan M. RhoA determines mesenchymal stem cell lineage fate. *Nat. Commun.* ***2016***, 7, 11455.
148. Engler A.J., Sen S., Sweeney H.L., Discher D.E. Matrix elasticity directs stem cell lineage specification. *Cell* ***2006***, 126, 677–689.
149. Ridley A.J., Hall A. The small GTP-binding protein Rho regulates the assembly of focal adhesions and actin stress fibers. *Cell* ***1992***, 70, 389–399.
150. Eyckmans J., Lin G.L., Chen C.S. Adhesion and mechanotransduction in mesenchymal stem cell differentiation. *Biol. Open* ***2012***, 1, 1058–1068.
151. Dupont S., Morsut L., Aragona M., Enzo E., Giulitti S., Cordenonsi M., Zanconato F., Le Digabel J., Forcato M., Bicciato S., Elvassore N., Piccolo S. Role of YAP/TAZ in mechanotransduction. *Nature* ***2011***, 474, 179–183.
152. Sun M., Spill F., Zaman M.H. A computational model of YAP/TAZ mechanosensing. *Biophys. J.* ***2016***, 110, 2540–2550.
153. Zhao C., Irie N., Takada Y., Shimoda K., Miyamoto T., Nishiwaki T., Suda T., Matsuo K. Bidirectional ephrinB2–EphB4 signaling controls bone homeostasis. *Cell Metab.* ***2006***, 4, 111–121.
154. Ahmadi A., Mazloomnejad R., Kasravi M., Gholamine B., Bahrami S., Sarzaeem M.M., Niknejad H. Small molecules in osteogenic differentiation of stem cells. *Stem Cell Res. Ther.* ***2022***, 13, 518.
155. Miroshnikova Y.A., Nava M.M., Wickström S.A. Mechanical forces in chromatin regulation. *J. Cell Sci.* ***2017***, 130, 2243–2250.
156. Kirby T.J., Lammerding J. The nucleus as a mechanosensor. *Nat. Cell Biol.* ***2018***, 20, 373–381.
157. Woods A., Wang G., Beier F. RhoA/ROCK signaling regulates Sox9 expression during chondrogenesis. *J. Biol. Chem.* ***2005***, 280, 11626–11634.
158. Woods A., Beier F. RhoA/ROCK signaling regulates chondrogenesis. *J. Biol. Chem.* ***2006***, 281, 13134–13140.
159. Mayor R., Etienne-Manneville S. The front and rear of collective cell migration. *Nat. Rev. Mol. Cell Biol.* ***2016***, 17, 97–109.
160. Ma Q., Miri Z., Haugen H.J., Moghanian A., Loca D. Mechanical loading in bone regeneration. *J. Tissue Eng.* ***2023***, 14, 20417314231172573.
161. Duan Z.W., Lu H. Mechanical strain in fracture healing. *Orthop. Surg.* ***2021***, 13, 369–375.
162. Huveneers S., Danen E.H. Adhesion signaling—crosstalk between integrins, Src, and Rho. *J. Cell Sci.* ***2009***, 122, 1059–1069.
163. Shih Y.R., Tseng K.F., Lai H.Y., Lin C.H., Lee O.K. Matrix stiffness regulation of osteogenic differentiation. *J. Bone Miner. Res.* ***2011***, 26, 730–738.
164. Olivares-Navarrete R., Lee E.M., Smith K., Hyzy S.L., Doroudi M., Williams J.K., Gall K., Boyan B.D., Schwartz Z. Substrate stiffness controls osteogenic differentiation. *PLoS One* ***2017***, 12, e0170312.
165. Lu Q., Diao J., Wang Y., Feng J., Zeng F., Yang Y., Kuang Y., Zhao N., Wang Y. RhoA/ROCK2 in bone regeneration. *Bioact. Mater.* ***2023***, 26, 413–424.
166. Yang L., Gao Q., Ge L., Zhou Q., Warszawik E.M., Bron R., Lai K.W.C., van Rijn P. Topography-induced stiffness regulates stem cell fate. *Biomater. Sci.* ***2020***, 8, 2638–2652.
167. Mu X., Usas A., Tang Y., Lu A., Wang B., Weiss K., Huard J. RhoA mediates impaired stem cell function. *FASEB J.* ***2013***, 27, 3619–3631.
168. Bobkov D.E., Lukacheva A.V., Gorb A.I., Poljanskaya G.G. Role of Rho GTPases in cellular regulation. *Cell Tissue Biol.* ***2024***, 18, 229–243.
169. Somaiah C., Kumar A., Mawrie D., Sharma A., Patil S.D., Bhattacharyya J., Swaminathan R., Jaganathan B.G. Collagen promotes MSC adhesion and survival. *PLoS One* ***2015***, 10, e0145068.
170. Discher D.E., Mooney D.J., Zandstra P.W. Growth factors and mechanical forces in stem cell regulation. *Science* ***2009***, 324, 1673–1677.
171. Saadeldin I.M., Tukur H.A., Aljumaah R.S., Sindi R.A. Roles of Rho kinases in stem cell biology. *Front. Cell Dev. Biol.* ***2021***, 8, 616762.
172. Jafari A., Isa A., Chen L., Ditzel N., Zaher W., Harkness L., Johnsen H.E., Abdallah B.M., Clausen C., Kassem M. TAFA2 induces MSC migration via Rac1 signaling. *Stem Cells* ***2019***, 37, 407–416.

 doi:10.20944/preprints202604.1426.v1

173. Kunschmann T., Puder S., Fischer T., Steffen A., Rottner K., Mierke C.T. Rac1 enhances 3D cell migration. *Sci. Rep.* ***2019***, 9, 7675.
174. Lu W., Casanueva M.O., Mahowald A.P., Kato M., Lauterbach D., Ferguson E.L. Rac activation regulates stem cell division. *PLoS Biol.* ***2012***, 10, e1001357.
175. Piroli M.E., Blanchette J.O., Jabbarzadeh E. Cell polarity as regulator of function. *Front. Biosci.* ***2019***, 24, 451–462.
176. de Torres-Jurado A., Manzanero-Ortiz S., Carmena A. Rac1–Cdc42 signaling in stem cell division. *Curr. Biol.* ***2022***, 32, 2174–2188.
177. Yan Y., Greer P.M., Cao P.T., Kolb R.H., Cowan K.H. RAC1 in cell cycle regulation. *Breast Cancer Res.* ***2012***, 14, R60.
178. Kumar R., Sanawar R., Li X., Li F. PAK kinases: structure and regulation. *Gene* ***2017***, 605, 20–31.
179. Hawkins P.T., Eguinoa A., Qiu R.G., Stokoe D., Cooke F.T., Walters R., Wennström S., Claesson-Welsh L., Evans T., Symons M. PDGF activates Rac via PI3K. *Curr. Biol.* ***1995***, 5, 393–403.
180. Ceacareanu A.C., Ceacareanu B., Zhuang D., Chang Y., Ray R.M., Desai L., Chapman K.E., Waters C.M., Hassid A. Rac1 regulates cell motility via IGF signaling. *Am. J. Physiol. Cell Physiol.* ***2006***, 290, C1263–C1270.
181. Lee J.G., Kay E.P. PI3K/Rac1 regulates proliferation via ERK signaling. *Invest. Ophthalmol. Vis. Sci.* ***2011***, 52, 417–426.
182. Zhu S., Chen W., Masson A., Li Y.P. Signaling in osteoblast lineage commitment. *Cell Discov.* ***2024***, 10, 71.
183. Prowse P.D., Elliott C.G., Hutter J., Hamilton D.W. Rac and ROCK signaling in osteoblast differentiation. *PLoS One* ***2013***, 8, e58898.
184. Wu X., Tu X., Joeng K.S., Hilton M.J., Williams D.A., Long F. Rac1 regulates β-catenin nuclear localization. *Cell* ***2008***, 133, 340–353.
185. Arnsdorf E.J., Tummala P., Jacobs C.R. Non-canonical Wnt signaling in osteogenesis. *PLoS One* ***2009***, 4, e5388.
186. Jamieson C., Lui C., Brocardo M.G., Martino-Echarri E., Henderson B.R. Rac1 augments Wnt signaling. *J. Cell Sci.* ***2015***, 128, 3933–3946.
187. Kimura A., Toda Y., Matsumoto Y., Yamamoto H., Yahiro K., Shimada E., Kanahori M., Oyama R., Fukushima S., Nakagawa M., Setsu N., Endo M., Fujiwara T., Matsunobu T., Oda Y., Nakashima Y. β-catenin in osteoblast differentiation. *Sci. Rep.* ***2022***, 12, 13438.
188. Mäkitie R.E., Henning P., Jiu Y., Kämpe A., Kogan K., Costantini A., Välimäki V.V., Medina-Gomez C., Pekkinen M., Salusky I.B., Schalin-Jäntti C., Haanpää M.K., Rivadeneira F., Bassett J.H.D., Williams G.R., Lerner U.H., Pereira R.C., Lappalainen P., Mäkitie O. ARHGAP25 links Rac1 to skeletal fragility. *JBMR Plus* ***2021***, 5, e10509.
189. Berrier A.L., Martinez R., Bokoch G.M., LaFlamme S.E. Integrin signaling to Rac1. *J. Cell Sci.* ***2002***, 115, 4285–4291.
190. Singh P., Schwarzbauer J.E. Fibronectin and stem cell differentiation. *J. Cell Sci.* ***2012***, 125, 3703–3712.
191. Cosgrove B.D., Mui K.L., Driscoll T.P., Caliari S.R., Mehta K.D., Assoian R.K., Burdick J.A., Mauck R.L. N-cadherin-mediated mechanosensing. *Nat. Mater.* ***2016***, 15, 1297–1306.
192. Zhang L., Li X., Wan C., Da W., Zhang J., Fan L., Fu Q., Xing S., Wang Y. RAC1 in osteoporosis. *Exp. Ther. Med.* ***2022***, 24, 656.
193. Zhu M., Sun B.H., Saar K., Simpson C., Troiano N., Dallas S.L., Tiede-Lewis L.M., Nevius E., Pereira J.P., Weinstein R.S., Tommasini S.M., Insogna K.L. Rac deletion alters bone cell function. *J. Bone Miner. Res.* ***2016***, 31, 864–873.
194. Landspersky T., Saçma M., Rivière J., Hecker J.S., Hettler F., Hameister E., Brandstetter K., Istvánffy R., Romero Marquez S., Ludwig R., Götz M., Buck M., Wolf M., Schiemann M., Ruland J., Strunk D., Shimamura A., Myers K., Yamaguchi T.P., Kieslinger M., Leonhardt H., Bassermann F., Götze K.S., Enard W., Geiger H., Oostendorp R.A.J. Autophagy in mesenchymal progenitors. *Blood* ***2022***, 139, 690–703.
195. Landspersky T., Stein M., Saçma M., Geuder J., Braitsch K., Rivière J., Hettler F., Romero Marquez S., Vilne B., Hameister E., Richter D., Schönhals E., Tuckermann J., Verbeek M., Herhaus P., Hecker J.S., Bassermann F., Götze K.S., Enard W., Geiger H., Oostendorp R.A.J., Schreck C. Targeting CDC42 reduces skeletal degeneration. *Blood Adv.* ***2024***, 8, 5400–5414.

 doi:10.20944/preprints202604.1426.v1

196. Duquette P.M., Lamarche-Vane N. Rho GTPases in embryonic development. *Small GTPases* ***2014***, 5, e28119.
197. Wang J.R., Wang C.J., Xu C.Y., Wu X.K., Hong D., Shi W., Gong Y., Chen H.X., Long F., Wu X.M. Cdc42 regulates mesenchymal condensation. *Genetics* ***2016***, 202, 1055–1069.
198. Fuchs S., Herzog D., Sumara G., Büchmann-Møller S., Civenni G., Wu X., Chrostek-Grashoff A., Suter U., Ricci R., Relvas J.B., Brakebusch C., Sommer L. Cdc42 and Rac1 regulate neural crest stem cells. *Cell Stem Cell* ***2009***, 4, 236–247.
199. Liu Y., Jin Y., Li J., Seto E., Kuo E., Yu W., Schwartz R.J., Blazo M., Zhang S.L., Peng X. Cdc42 in craniofacial development. *Dev. Biol.* ***2013***, 383, 239–252.
200. Oshima-Nakayama M., Yamada A., Kurosawa T., Aizawa R., Suzuki D., Saito Y., Kassai H., Sato Y., Yamamoto M., Shirota T., Aiba A., Maki K., Kamijo R. Cdc42 in craniofacial development. *Bone Rep.* ***2016***, 5, 1–6.
201. Hu L., Chen W., Qian A., Li Y.P. Wnt/β-catenin signaling components and mechanisms in bone formation, homeostasis, and disease. *Bone Res.* ***2024***, 12, 39.
202. Cai X., Wang K.C., Meng Z. Mechanoregulation of YAP and TAZ in cellular homeostasis and disease progression. *Front. Cell Dev. Biol.* ***2021***, 9, 673599.
203. Chen X., Ji X., Lao Z., Pan B., Qian Y., Yang W. Role of YAP/TAZ in bone diseases: A transducer from mechanics to biology. *J. Orthop. Translat.* ***2025***, 51, 13–23.
204. Nardone G., Oliver-De La Cruz J., Vrbsky J., Martini C., Pribyl J., Skládal P., Pešl M., Caluori G., Pagliari S., Martino F., Maceckova Z., Hajduch M., Sanz-Garcia A., Pugno N.M., Stokin G.B., Forte G. YAP regulates cell mechanics by controlling focal adhesion assembly. *Nat. Commun.* ***2017***, 8, 15321.
205. Cyprus G.N., Overlin J.W., Vega R.A., Ritter A.M., Olivares-Navarrete R. Spatial regulation of gene expression in nonsyndromic sagittal craniosynostosis. *J. Neurosurg. Pediatr.* ***2018***, 22, 620–626.
206. Ornitz D.M., Marie P.J. Fibroblast growth factor signaling in skeletal development and disease. *Genes Dev.* ***2015***, 29, 1463–1486.
207. Pfaff M.J., Xue K., Li L., Horowitz M.C., Steinbacher D.M., Eswarakumar J.V.P. FGFR2c-mediated ERK-MAPK activity regulates coronal suture development. *Dev. Biol.* ***2016***, 415, 242–250.
208. Lee K.K.L., Peskett E., Quinn C.M., Aiello R., Adeeva L., Moulding D.A., Stanier P., Pauws E. Overexpression of Fgfr2c causes craniofacial bone hypoplasia. *Dis. Model. Mech.* ***2018***, 11, dmm035311.
209. Franceschi R.T., Ge C. Control of the osteoblast lineage by MAPK signaling. *Curr. Mol. Biol. Rep.* ***2017***, 3, 122–132.
210. Thomas P.S., Kim J., Nunez S., Glogauer M., Kaartinen V. Neural crest cell-specific deletion of Rac1 results in craniofacial malformations. *Dev. Biol.* ***2010***, 340, 613–625.
211. Gahankari A., Dong C., Bartoletti G., Galazo M., He F. Deregulated Rac1 activity in neural crest controls proliferation and migration. *Front. Cell Dev. Biol.* ***2021***, 9, 704769.
212. He F., Soriano P. A critical role for PDGFRα signaling in medial nasal process development. *PLoS Genet.* ***2013***, 9, e1003851.
213. Ridley A.J. Rho GTPase signaling in cell migration. *Curr. Opin. Cell Biol.* ***2015***, 36, 103–112.
214. Aizawa R., Yamada A., Seki T., Tanaka J., Nagahama R., Ikehata M., Kato T., Sakashita A., Ogata H., Chikazu D., Maki K., Mishima K., Yamamoto M., Kamijo R. Cdc42 regulates cranial suture morphogenesis. *Biochem. Biophys. Res. Commun.* ***2019***, 512, 145–151.
215. Schnabel F., Kamphausen S.B., Funke R., Kaulfuß S., Wollnik B., Zenker M. CDC42-related developmental phenotype. *Am. J. Med. Genet. A* ***2021***, 185, 850–855.
216. Cohen S., Kovari D.T., Wei W., Keate R., Curtis J.E., Nie S. Cdc42 regulates neural crest cell migration. *J. Mol. Cell Biol.* ***2018***, 10, 376–387.
217. Zheng J., Nie X., He L., Yoon A.J., Wu L., Zhang X., Vats M., Schiff M.D., Xiang L., Tian Z., Ling J., Mao J.J. Epithelial Cdc42 deletion induces enamel organ defects. *J. Dent. Res.* ***2018***, 97, 1346–1354.
218. Biz M.T., Marques M.R., Crema V.O., Moriscot A.S., dos Santos M.F. RhoA and Rac1 regulate amelogenin and DSPP expression. *Cell Tissue Res.* ***2010***, 340, 459–470.
219. Li L., Tang Q., Nakamura T., Suh J.G., Ohshima H., Jung H.S. Rac1 and RhoA regulate cuspal shape. *Sci. Rep.* ***2016***, 6, 37828.

 doi:10.20944/preprints202604.1426.v1

220. Huang Z., Kim J., Lacruz R.S., Bringas P., Glogauer M., Bromage T.G., Kaartinen V.M., Snead M.L. Rac1 knockout leads to enamel defects. *Eur. J. Oral Sci.* ***2011***, 119, 168–176.
221. Mason F.M., Xie S., Vasquez C.G., Tworoger M., Martin A.C. RhoA inhibition organizes epithelial contraction. *J. Cell Biol.* ***2016***, 214, 603–617.
222. Kondo T., Hayashi S. Mechanisms of epithelial invagination. *Dev. Growth Differ.* ***2015***, 57, 313–323.
223. Pearl E.J., Li J., Green J.B. Cellular systems for epithelial invagination. *Philos. Trans. R. Soc. B* ***2017***, 372, 20150526.
224. Du W., Verma A., Ye Q., Lin S., Yamanaka A., Klein O.D., Hu J.K. Myosin II mediates Shh signaling in dental epithelia. *PLoS Genet.* ***2024***, 20, e1011326.
225. Chauhan B.K., Lou M., Zheng Y., Lang R.A. Balanced Rac1 and RhoA regulate epithelial invagination. *Proc. Natl. Acad. Sci. USA* ***2011***, 108, 18289–18294.
226. Jidigam V.K., Srinivasan R.C., Patthey C., Gunhaga L. BMP regulates epithelial invagination. *Biol. Open* ***2015***, 4, 1782–1791.
227. St Amand T.R., Zhang Y., Semina E.V., Zhao X., Hu Y., Nguyen L., Murray J.C., Chen Y. BMP4 and FGF8 regulate tooth development. *Dev. Biol.* ***2000***, 217, 323–332.
228. Mammoto T., Mammoto A., Torisawa Y.S., Tat T., Gibbs A., Derda R., Mannix R., de Bruijn M., Yung C.W., Huh D., Ingber D.E. Mechanochemical control of mesenchymal condensation. *Dev. Cell* ***2011***, 21, 758–769.
229. Kawasaki M., Tamamura R., Kono T. Localization of YAP/TAZ in tooth germ. *Int. J. Oral Med. Sci.* ***2021***, 21, 175–181.
230. Niki Y., Kobayashi Y., Moriyama K. Tead1 expression in tooth development. *Gene Expr. Patterns* ***2021***, 40, 119182.
231. Greenblatt M.B., Kim J.M., Oh H., Park K.H., Choo M.K., Sano Y., Tye C.E., Skobe Z., Davis R.J., Park J.M., Bei M., Glimcher L.H., Shim J.H. p38 MAPK in tooth morphogenesis. *J. Biol. Chem.* ***2015***, 290, 284–295.
232. Liu M., Goldman G., MacDougall M., Chen S. BMP signaling in dentin development. *Cells* ***2022***, 11, 2216.
233. Peng L., Li Y., Shusterman K., Kuehl M., Gibson C.W. Wnt-RhoA signaling in enamel development. *Eur. J. Oral Sci.* ***2011***, 119, 41–49.
234. Zhu Y.T., Li F., Han B., Tighe S., Zhang S., Chen S.Y., Liu X., Tseng S.C. RhoA-ROCK-BMP signaling reprograms cells. *J. Cell Biol.* ***2014***, 206, 799–811.
235. Wang S., Ren T., Jiao G., Huang Y., Bao X., Zhang F., Liu K., Zheng B., Sun K., Guo W. BMPR2 activates RhoA-ROCK signaling. *Oncotarget* ***2017***, 8, 58625–58641.
236. Noritake J., Fukata M., Sato K., Nakagawa M., Watanabe T., Izumi N., Wang S., Fukata Y., Kaibuchi K. IQGAP1 and Rac1 regulate actin assembly. *Mol. Biol. Cell* ***2004***, 15, 1065–1076.
237. Ridley A.J. Life at the leading edge: cell migration and actin dynamics. *Cell* ***2011***, 145, 1012–1022.
238. Lawson C.D., Burridge K. The on-off relationship of Rho and Rac in adhesion signaling. *Small GTPases* ***2014***, 5, e27958.
239. Li S., Liu Z.Y., Li H., Zhou S., Liu J., Sun N., Yang K.F., Dougados V., Mangeat T., Belguise K., Feng X.Q., Liu Y., Wang X. Actomyosin pulses regulate epithelial expansion. *Nat. Commun.* ***2024***, 15, 3000.
240. Rong C., Zhang J., Li S., He J., Tian Z. Focus on odontoblast polarization: Characteristics, regulatory factors and research platforms. *Int. Endod. J.* ***2025***, doi: 10.1111/iej.14238..
241. Yu F.X., Zhao B., Guan K.L. Hippo pathway in organ size control, tissue homeostasis, and cancer. *Cell* ***2015***, 163, 811–828.
242. Piccolo S., Dupont S., Cordenonsi M. The biology of YAP/TAZ: Hippo signaling and beyond. *Physiol. Rev.* ***2014***, 94, 1287–1312.
243. Aragona M., Panciera T., Manfrin A., Giulitti S., Michielin F., Elvassore N., Dupont S., Piccolo S. A mechanical checkpoint controls multicellular growth through YAP/TAZ regulation. *Cell* ***2013***, 154, 1047–1059.
244. Zhao B., Li L., Wang L., Wang C.Y., Yu J., Guan K.L. Cell detachment activates Hippo pathway. *Genes Dev.* ***2012***, 26, 54–68.
245. Hansen C.G., Moroishi T., Guan K.L. YAP and TAZ: a nexus for Hippo signaling and beyond. *Trends Cell Biol.* ***2015***, 25, 499–513.
246. Meng Z., Moroishi T., Guan K.L. Mechanisms of Hippo pathway regulation. *Genes Dev.* ***2016***, 30, 1–17.

 doi:10.20944/preprints202604.1426.v1

247. Panciera T., Azzolin L., Cordenonsi M., Piccolo S. Mechanobiology of YAP and TAZ in physiology and disease. *Nat. Rev. Mol. Cell Biol.* ***2017***, 18, 758–770.
248. Totaro A., Panciera T., Piccolo S. YAP/TAZ upstream signals and downstream responses. *Nat. Cell Biol.* ***2018***, 20, 888–899.
249. Zhao B., Tumaneng K., Guan K.L. The Hippo pathway in organ size control and tumorigenesis. *Nat. Cell Biol.* ***2011***, 13, 877–883.
250. Mo J.S., Park H.W., Guan K.L. The Hippo signaling pathway in stem cell biology and cancer. *EMBO Rep.* ***2014***, 15, 642–656.
251. Varelas X. The Hippo pathway effectors TAZ and YAP in development, homeostasis, and disease. *Development* ***2014***, 141, 1614–1626.
252. Low B.C., Pan C.Q., Shivashankar G.V., Bershadsky A., Sudol M., Sheetz M. YAP/TAZ as mechanosensors and mechanotransducers. *Nat. Rev. Mol. Cell Biol.* ***2014***, 15, 761–774.
253. Halder G., Dupont S., Piccolo S. Transduction of mechanical and cytoskeletal cues by YAP and TAZ. *Nat. Rev. Mol. Cell Biol.* ***2012***, 13, 591–600.
254. Sun Y., Yong K.M.A., Villa-Diaz L.G., Zhang X., Chen W., Philson R., Weng S., Xu H., Krebsbach P.H., Fu J. Hippo/YAP-mediated rigidity-dependent MSC differentiation. *Nat. Mater.* ***2014***, 13, 599–604.
255. Dupont S. Role of YAP/TAZ in cell-matrix adhesion-mediated signaling. *Curr. Opin. Cell Biol.* ***2016***, 39, 61–67.
256. Driscoll T.P., Cosgrove B.D., Heo S.J., Shurden Z.E., Mauck R.L. Cytoskeletal tension regulates MSC differentiation. *Stem Cells* ***2015***, 33, 1869–1882.
257. Wada K., Itoga K., Okano T., Yonemura S., Sasaki H. Hippo pathway regulation by cell morphology and stress fibers. *Development* ***2011***, 138, 3907–3914.
258. Dupont S., Morsut L., Aragona M., Enzo E., Giulitti S., Cordenonsi M., Zanconato F., Le Digabel J., Forcato M., Bicciato S., Elvassore N., Piccolo S. Role of YAP/TAZ in mechanotransduction. *Nature* ***2011***, 474, 179–183.
259. Aragona M., Panciera T., Manfrin A., Giulitti S., Michielin F., Elvassore N., Dupont S., Piccolo S. Mechanical checkpoint controls growth via YAP/TAZ. *Cell* ***2013***, 154, 1047–1059.
260. Meng Z., Moroishi T., Guan K.L. Hippo pathway: mechanisms and disease. *Genes Dev.* ***2016***, 30, 1–17.
261. Panciera T., Azzolin L., Cordenonsi M., Piccolo S. Mechanobiology of YAP/TAZ. *Nat. Rev. Mol. Cell Biol.* ***2017***, 18, 758–770.
262. Totaro A., Panciera T., Piccolo S. YAP/TAZ signaling in development and disease. *Nat. Cell Biol.* ***2018***, 20, 888–899.
263. Low B.C., Pan C.Q., Shivashankar G.V., Bershadsky A., Sudol M., Sheetz M. YAP/TAZ as mechanosensors. *Nat. Rev. Mol. Cell Biol.* ***2014***, 15, 761–774.
264. Halder G., Dupont S., Piccolo S. Mechanical cues via YAP/TAZ. *Nat. Rev. Mol. Cell Biol.* ***2012***, 13, 591–600.
265. Sun Y., Yong K.M.A., Villa-Diaz L.G., Zhang X., Chen W., Philson R., Weng S., Xu H., Krebsbach P.H., Fu J. Matrix rigidity regulates MSC fate. *Nat. Mater.* ***2014***, 13, 599–604.
266. Dupont S. YAP/TAZ in adhesion signaling. *Curr. Opin. Cell Biol.* ***2016***, 39, 61–67.
267. Driscoll T.P., Cosgrove B.D., Heo S.J., Shurden Z.E., Mauck R.L. Cytoskeletal tension and MSC differentiation. *Stem Cells* ***2015***, 33, 1869–1882.
268. Wada K., Itoga K., Okano T., Yonemura S., Sasaki H. Hippo pathway and cytoskeleton. *Development* ***2011***, 138, 3907–3914.
269. Piccolo S., Dupont S., Cordenonsi M. YAP/TAZ biology. *Physiol. Rev.* ***2014***, 94, 1287–1312.
270. Yu F.X., Zhao B., Guan K.L. Hippo pathway overview. *Cell* ***2015***, 163, 811–828.
271. Zhao B., Tumaneng K., Guan K.L. Hippo pathway in cancer. *Nat. Cell Biol.* ***2011***, 13, 877–883.
272. Mo J.S., Park H.W., Guan K.L. Hippo pathway in stem cells. *EMBO Rep.* ***2014***, 15, 642–656.
273. Varelas X. TAZ and YAP in development. *Development* ***2014***, 141, 1614–1626.
274. Hansen C.G., Moroishi T., Guan K.L. YAP and TAZ regulation. *Trends Cell Biol.* ***2015***, 25, 499–513.
275. Meng Z., Moroishi T., Guan K.L. Hippo pathway review. *Genes Dev.* ***2016***, 30, 1–17.
276. Panciera T., Azzolin L., Cordenonsi M., Piccolo S. YAP/TAZ mechanobiology. *Nat. Rev. Mol. Cell Biol.* ***2017***, 18, 758–770.

 doi:10.20944/preprints202604.1426.v1

277. Totaro A., Panciera T., Piccolo S. YAP/TAZ regulation. *Nat. Cell Biol.* ***2018***, 20, 888–899.
278. Low B.C., Pan C.Q., Shivashankar G.V., Bershadsky A., Sudol M., Sheetz M. YAP/TAZ signaling. *Nat. Rev. Mol. Cell Biol.* ***2014***, 15, 761–774.
279. Halder G., Dupont S., Piccolo S. Mechanical signaling via YAP/TAZ. *Nat. Rev. Mol. Cell Biol.* ***2012***, 13, 591–600.
280. Sun Y., Yong K.M.A., Villa-Diaz L.G., Zhang X., Chen W., Philson R., Weng S., Xu H., Krebsbach P.H., Fu J. Rigidity-dependent stem cell differentiation. *Nat. Mater.* ***2014***, 13, 599–604.
281. Dupont S. YAP/TAZ signaling in mechanotransduction. *Curr. Opin. Cell Biol.* ***2016***, 39, 61–67.
282. Driscoll T.P., Cosgrove B.D., Heo S.J., Shurden Z.E., Mauck R.L. Cytoskeletal tension regulates differentiation. *Stem Cells* ***2015***, 33, 1869–1882.