OPEN ACCESS Freely available online

Journal of Bone Research

Review Article

A Review of Dysregulated Osteoblast and Osteoclast Coupling in Bone Disease and Failure

Richard Visconti[1#], Timothy Iversen[1#], Jessica Cottrell[1*]

Seton Hall University, 400 S Orange Ave, South Orange, NJ 07079, USA

[#]Both authors contributed equally to this work.

ABSTRACT

Human bones are formed through intramembranous and endochondral ossification followed by a period of appositional growth. Skeletal homeostasis of cancellous/trabecular and cortical bone tissue is sustained through a lifelong biological process known as bone remodeling. Bone remodeling is the balanced-integrated function of osteocyte signaling, osteoblast bone formation, and osteoclast bone resorption. In this review, the autocrine and paracrine factors that control the rate of bone synthesis and resorption as they attribute to osteogenic cell differentiation, localization, and function are reviewed. These factors direct the transition between each phase of the remodeling process: activation, resorption, reversal, formation, and mineralization. The five primary intracellular signaling pathways that regulate osteogenic gene expression, cell function, localization, and survival include: Wnt/β-catenin, transforming growth factor-β, bone morphogenetic protein, arachidonic acid metabolism/prostaglandin synthesis, and receptor activator of nuclear factor κ B are also discussed. Several diseases are associated with dysregulated bone remodeling and aberrant signaling in osteogenic cells. Some hereditable and acquired genetic mutations result in skeletal diseases, like craniometaphyseal dysplasia, osteogenesis imperfecta, osteopetrosis, and myeloma bone disease. Other skeletal disorders are attributed to endogenous and exogenous induced hormonal imbalances, like postmenopausal osteoporosis or glucocorticoid steroid use, or cytokine imbalances that exacerbate inflammatory diseases, like rheumatoid arthritis. The role of excessive resorption and inadequate bone formation have in these diseases that may result in overall decreased skeletal tissue integrity, chronic pain, pathological bone fractures, and mortality are also examined.

Keywords: Osteoblast; Osteoclast; Osteocyte; Osteogenic; Remodeling; Resorption; Mineralization; Hydroxyapatite

INTRODUCTION

Normal bone homeostasis mechanisms

The orchestrated function of osteocytes, osteoblasts, and osteoclasts within a bone remodeling cavity, Howship's lacunae, was defined as the Basic Multicellular Unit (BMU) by Frost in the 1960s [1]. Normal and balanced coupling leads to a remodeling cycle that lasts approximately 120 days for cortical bone and over 200 days for trabecular/cancellous bone [2]. A US Surgeon General's report on bone health and osteoporosis issued in 2004 stated that the human skeleton is replaced every 10 years [3].

Normal bone remodeling is initiated through osteoclast activation by means of direct mechanical stress/structural impairment or hormonal signaling, estrogen or parathyroid hormone (PTH). Osteoclastogenesis begins when Bone Marrow Macrophages (BMM) exposed to granulocyte-macrophage colony-stimulating factor (GM-CSF) form multinucleated osteoclasts that express receptor activator of nuclear factor κ B (RANK). Osteocytes may undergo apoptosis or secrete sclerostin, a Wingless/Integrated (Wnt) and bone morphogenetic protein

Correspondence to: Jessica Cottrell Seton Hall University, 400 S Orange Ave, South Orange, NJ 07079, USA, E-mail: jessica.cottrell@shu.edu

Received: August 20, 2019; **Accepted:** September 1, 2019; **Published:** September 7, 2019

Citation: Visconti R, et al. (2019) A Review of Dysregulated Osteoblast and Osteoclast Coupling in Bone Disease and Failure. J Bone Res 7:201. doi:10.35248/2572-4916.19.7.201.

(BMP) antagonist that suppresses osteoblastogenesis, and RANKL that binds RANK receptor on osteoclasts to promote differentiation and survival signals within the cells [4]. Osteoblasts also regulate osteoclastogenesis through secretion of osteoprotegerin, a soluble RANKL decoy receptor [5]. Bone metabolism is highly regulated by vitamin D, PTH, interleukin-1 (IL-1), and prostaglandins [6].

The resorptive phase of bone remodeling starts when osteoclasts localize to the bone surface, attach via podosomes, polarize, seal, and secrete hydrogen ions (H+) to acidify the resorption site and expose the bone tissue's Extracellular Matrix (ECM) [7]. Osteoclasts also secrete cathepsin K, a protease that catabolizes collagen, elastin, and gelatin, that denudes the remaining matrix and forms the resorption lacunae [8]. The proteolytic decomposition of the ECM liberates signaling molecules, like Transforming Growth Factor β (TGF-β) embedded in the matrix by osteoblasts during bone formation. The entire resorption phase of bone remodeling takes approximately 3 weeks in humans [9].

The reversal phase, which takes approximately 9 days, is a transitional period where resorption activity is halted, and the bone microenvironment becomes conducive for osteoblastic formation. During a reversal, three primary events take place: osteoclasts undergo apoptosis, peripheral macrophages clean the resorption zone and prime it for new bone deposition, and osteoblasts differentiate and localize to the denuded bone's resorption site, Howship's lacunae [7].

Bone formation or modeling occurs when Wnt/β-catenin, TGF-β, and BMP induces osteoblastogenesis where Bone Marrow Stromal Cells (BMSC) differentiate into pre-osteoblasts and mature into functional osteoblasts. The mature osteoblasts localize to the denuded bone and secrete osteoid, the unmineralized organic portion of bone primarily composed of type I collagen, chondroitin sulfate, and osteocalcin [10]. Bone formation is a protracted process that takes several months to complete [11].

The bone tissue extracellular matrix is complete when the osteoblasts cause calcium and phosphate salts, known as hydroxyapatite or $Ca_{10}(PO_4)_6(OH)_2$, to precipitate from the blood and mineralize the matrix. Osteoblasts also secrete alkaline phosphatase, a ubiquitous homodimeric enzyme essential for bone mineralization. New osteocytes are formed when osteoblasts become embedded in the ECM and form lacunae and canaliculi. Osteocytes comprise over 90% of the cellular component of bone, approximately 42 billion in the adult human skeleton [12]. Osteocyte's mechanosensory signaling networks play a major role in activating osteoclasts and osteoblasts during multiple phases of bone remodeling.

Once the newly formed bone has been mineralized and is replete with osteocytes the bone remodeling process enters a quiescent resting phase. Unlike osteoclasts and osteoblasts that have average lifespans of 2 weeks and 3 months respectively, osteocytes can live for 25 years [13]. Microfractures created through normal daily activity and exercise, bone trauma, vitamin deficiency, altered hormone-bone signaling, and genetic skeletal diseases induce active bone remodeling [14,15].

Fracture healing bone homeostasis

Bone tissue regeneration involves a multitude of concurrent processes to connect fracture terminals and to restore the physiological function of damaged bone. The reclamation of cellular biochemical properties and the reestablishment of the tissue's biomechanical integrity terminates the restorative process. Solheim categorizes the stages of healing in four major components: immediate injury response, intramembranous bone formation, chondrogenesis, and endochondral ossification [16]. Within the cascade of stepwise phases, the growth factors BMP-2/4, TGF-β, aFGF, bFGF, and IGF-I are released respectively to the cellular intermediates within the cascade and are responsible for propagating the regenerative process.

The vascularization of bone tissue stages the initial response to fracture. The migration of macrophages and neutrophils to the fracture site introduces the onset of inflammation. These immune cells work to degrade cellular debris, foreign pathogens, and other unnecessary presences surrounding the trauma. The immune response begins the restorative process, allowing for effective growth factor signaling and the traversement of regenerative phases.

O'Connor and Lysz outline the regenerative phases, presenting the rapid proliferation of periosteoblasts and the migration of Mesenchymal Stem Cells (MSCs) at the fracture site first [17]. MSCs meet the proliferating osteoblasts to form a callus at the bridge ends of the bone. Post callus formation, MSCs differentiate into chondrocytes; acting to form a matrix of cartilage [18]. This cartilaginous matrix then becomes calcified after chondrocytes become hypertrophic and self-induce apoptosis. The osteoblasts then form bone, using the calcified cartilage-matrix as a scaffold.

Angiogenesis, the process of forming new capillaries and vasculature to de novo tissues, is integral to the delivery of osteoclasts, osteoblasts, necessary nutrients, and signaling intermediates to the calcified matrix. Osteoclasts then breakdown and resorb the calcified matrix along with the newly formed bone. Osteoblasts, working in conjunction with osteoclasts, then replace the resorbed bone with a mature bone until both sides of the fracture meet. The bone ends fuse with continued remodeling until the normal physiological and biochemical properties of the bone have been reestablished. The stepwise remodeling of post-fracture bone is not as arbitrary as a brief synopsis may be perceived. There are many signaling pathways and molecular intermediates that influence bone homeostasis and fracture healing.

Key pathways that regulate skeletal homeostasis

Wnt/β-catenin signaling: Wingless/Integrated (Wnt) signaling is an ancient biological process that is evolutionary conserved from Drosophila melanogaster to Homo sapiens. It consists of three distinct pathways: canonical Wnt, canonical planar cell polarity, and noncanonical Wnt/calcium signaling mechanisms. Wnt signaling is ubiquitously found throughout the human physiology and is responsible for stem cell potency, proliferation, function, and differentiation.

The canonical Wnt pathway is critical for tissue renewal, as demonstrated in the intestinal epithelium, and tissue repair, like hepatic regeneration [19]. In human bone remodeling, canonical Wnt signaling plays an integral role in osteogenic differentiation in mesenchymal stem cells while suppressing concomitant chondrogenic and adipogenic lineages. The signal is primarily responsible for the initiation of osteoblastogenesis and continues through osteoprogenitor proliferative stage, preosteoblast matrix formation, osteoblast maturation and mineralization, and osteocyte formation [20].

When Wnt receptors are unbound the destruction complex, consisting of Ser/Thr kinases Glycogen Synthase Kinase 3 (GSK-3) and Casein Kinase 1 (CK1), the scaffolding protein Axin, the Adenomatous Polyposis Coli (APC) protein, and the E3-ubiquitin ligase β-TrCP, phosphorylates β-catenin and ultimately leads to proteasomal degradation of the protein [21]. Canonical Wnt signaling is initiated when a R-spondin, a family of secreted proteins that contain two N-terminal furin domains and a thrombospondin domain, binds to a Leucine-Rich repeat-containing G-Protein Coupled Receptor (LGR) expressed on the cell membrane. The ligand-bound receptor interacts with the coreceptor, Frizzled, causing components of the destruction complex to relocate to the membrane. This interaction dissociates APC from the destruction complex and inhibits GSK3 phosphorylation of β-catenin [22]. This causes an accumulation of β-catenin in the cytoplasm that eventually translocates into the nucleus where it serves as transcription factors that promote osteogenesis.

In Bone Marrow Stromal Cells (BMSC) nuclear translocation of β-catenin and subsequent interactions with T Cell Factor (TCF)-1 form a complex that binds the Runt-Related Transcription Factor 2 (RUNX2) promoter [23]. RUNX2 transcription commits BMS/MSCs to the osteogenic lineage as preosteoblasts. RUNX2 mutations have been associated with critical defects in bone formation and in cleidocranial dysplasia, defective development of the cranial bones and complete or partial absence of the clavicles [24]. BMP-2 is also essential in RUNX2 activation and osteogenic cell fate determination and exemplifies the importance of crosstalk between different signaling pathways in bone remodeling.

The interplay between canonical Wnt and BMP signaling plays a critical role in osteoblast maturation as well [25]. During mineralization osteoblast expression and secretion of Alkaline Phosphatase (AP), a metalloenzyme critical in inorganic phosphate formation and calcification, is β-catenin-dependent [26]. Bone Alkaline Phosphatase (BAP) hydrolyzes Inorganic Pyrophosphate (PPi), a mineralization inhibitor, and generates inorganic phosphate (Pi), an essential component of hydroxyapatite formation [27]. An inverse correlation between low bone mineral density associated with certain metabolic bone diseases, like diabetes, and higher levels of bone alkaline phosphatase has also been reported. This BAP activity is believed to be corrective in nature and does not suggest an inhibitory role of BAP on bone mineralization in diabetics [28].

Canonical Wnt signaling is crucial in osteoblastic regulation of osteoclastogenesis. Osteoblasts express at least three genes that regulate osteoclast differentiation: Receptor Activator Of Nuclear Factor K-B Ligand (RANKL), Osteoprotegerin (Opg), and Macrophage Colony-Stimulating Factor (M-CSF). Both RANKL and Opg are β-catenin dependent genes under the control of the canonical Wnt pathway. RANKL, a member of the Tumor Necrosis Factor (TNF) superfamily, is a type II membrane protein that controls osteoclast cell proliferation by modifying levels of DNA-binding protein inhibitors Id2, Id4, and cyclin-D1. Opg, also a TNF superfamily member, is an osteoclastogenesis inhibitor that acts as a decoy receptor for RANKL [29]. Inhibition of Wnt/β-catenin signaling plays a crucial role in proper heart, head, and forelimb development during anterior morphogenesis of the embryo [30]. Dickkopf Wnt Signaling Pathway Inhibitor 1 (Dkk1) is a secreted protein that binds LGRs making them inaccessible to Wnt agonists, like R-spondins, halting bone remodeling.

Canonical Wnt signaling is considered a valuable target in the treatment of several human skeletal disorders. Irregularities in Wnt signaling are associated with multiple myeloma, osteoporosis, osteoporosis-pseudoglioma syndrome, sclerosteosis, Van Buchem disease, and rheumatoid arthritis.

TGF-β signaling: Transforming Growth Factor Beta (TGF-β) is a multifunctional cytokine that includes three different mammalian isoforms: TGF-β1, TGF-β2, and TGF-β3 [31]. The cytokine plays an integral part in the osteoclastogenesis of Bone Marrow Macrophages (BMM) and contributes to the regulation of osteoblastogenesis [32]. The TGF-β complex consists of propeptide and homodimer regions, the latter of which, interacts with a Latency Associated Protein (LAP) that forms the Small Latent Product (SLP). SLP remains in the osteoblast until bound by Latent TGF-β-Binding Protein (LTBP) converts the SLP into the Large Latent Protein (LLP), the latent TGF-β product deposited in the Extracellular Matrix (ECM) of bone [33]. LLP remains in the ECM until it is released and converted to active TGF-β during bone resorption by osteoclasts [34]. Low levels of active TGF-β induce macrophage migration, conversely, high levels of active TGF-β inhibit localization of osteoclast precursors to the resorption site [31].

The unbound active TGF-β plays several roles in osteoblasts by inducing osteoclastogenesis through increasing RANKL expression, suppressing proliferation and differentiation through Small worm phenotype and mothers against decapentaplegic (Smad)-mediated inhibition of pro-osteoblastogenic genes like RUNX2 and by inducing osteoblastogenesis through Non-Smad p38/Mitogen-Activated Protein Kinase (MAPK) signaling [35]. Although precise control over these contradictory, signaling mechanisms remains unclear, some have suggested that BMP receptors regulate intracellular TGF-β signaling [36,37].

More specifically, the active TGF-β binds the tetrameric receptor complex, which consists of two type I and two type II receptors and elicits Type II receptor endocytosis and transphosphorylation by the Smad Anchor for Receptor Activation (SARA). The phosphorylated Receptor-regulated Smad (R-Smad), consisting of a Smad2/Smad3, interacts with Smad4 to form a complex that translocates into the nucleus where it recruits Histone Deacetylases (HDAC) to inhibit RUNX2 expression [35].

In non-Smad signaling, receptor activations lead to TGF-β Activated Kinase 1 (TAK1) phosphorylation. TAK1 recruits TAK1-Binding protein 1 (TAB1) and initiates p38 MAPK pathway by serving as a scaffold protein and activator of p38 [38]. Upon activation p38 phosphorylates RUNX2 protein enhancing its association with Creb-Binding Protein (CBP), promoting expression of osteoblastic genes [39].

Elevated levels of TGF-β1 have been found throughout the normal fracture healing process [40]. This result was expected since TGF-βs regulate osteogenic chemotaxis and osteoblastic gene regulation. Irregular TGF-β signaling has been associated with delayed fracture healing and metabolic bone disorders [41]. Higher levels of TGF-β have been found in the serum and bone tissue of people suffering from chronic kidney disease [42]. Disproportionate TGF-βsignaling plays a role in osteogenesis imperfecta [43] and gene polymorphisms have been tied to susceptibility to postmenopausal osteoporosis [44].

BMP signaling: BMP members of the TGF-β superfamily, are a group of cytokines that regulate cell growth and differentiation. They were aptly named for their ability to induce bone and cartilage formation. BMPs play a critical role in embryonic skeletal development, endochondral ossification, bone remodeling, and bone repair [45]. BMP2 and BMP4 gene knockouts prove lethal in the embryo, whereas BMP1, BMP7, and BMP11 knockouts are extremely short-lived [46]. Much like TGF-β signaling, BMP signaling occurs through tetrameric binding of Type I and Type II receptors followed by Smad-dependent and Smad-independent pathways [31] and has dual roles in bone remodeling.

On the cell's plasma membrane, BMP binds the BMP receptor's GS domain initiates Smad-mediated signaling. The type II receptor activates the kinase activity of the type I receptor which leads to the phosphorylation of the R-smad 1/5/8. This phosphorylation event occurs through the interaction between the MH2 domain on the Type I receptor and Rsmad 1/5/8 [37]. The BMP receptor dissociates from the phosphorylated Rsmad freeing it to translocate to the nucleus and influence BMP-related gene expression directly or indirectly through interactions with DNA-binding proteins [37].

BMP also initiates Smad-independent signaling in a manner like TGF-β, but MAPK phosphorylation targets the osteoblast differentiation stimulator, Dlx5 and the osteogenesis activator Osterix (Osx) in addition to Runx2. The non-canonical BMP cascade relies on TAK1 phosphorylation and ultimately leads to upregulation of Runx2 osteogenic target genes. The smad-independent BMP works in conjunction with the Smad-dependent pathway to regulate bone formation during limb development [47].

Crosstalk between the BMP and Wnt/β-catenin signaling pathways exemplifies the duality of BMP signaling in bone remodeling. BMP signaling increases expression of Wnt antagonists, DKK1 and Sclerostin [47]. Conversely, BMP enhances Wnt signaling by forming a co-transcriptional complex with β-catenin eliciting increased RUNX2 expression [48].

BMP signaling is also regulated through BMP receptor antagonization through several cognate binding proteins: Noggin, Chordin, Gremlin, and Follistatin [49]. These BMP inhibitors are key regulators of bone developmental progression and play an essential role during the later chondrogenic-osteogenic transition by binding BMP cognate receptors [49]. This process is essential in skeletanogenesis and missense mutations in Noggin have been associated with the disorders of proximal symphalangism and multiple synostoses syndrome [50].

BMP mutations and dysregulation are associated with several bone diseases. Fibrodysplasia ossificans progressiva, progressive heterotropic ossification, results from a heterozygous missense mutation in a BMP type I receptor, activin receptor IA [51]. It has also been reported that osteoporotic humans and rats suffering from delayed fracture healing had significantly lower levels of BMP-2 than their respective normal cohorts [52]. Others have published data showing decreased levels of BMP-9 in Type 2 diabetics, suggesting that the BMP-9 deficiency plays an integral role in insulin resistance [53].

Arachidonic acid/metabolites pathway: Prostaglandins are Arachidonic Acid (AA) lipid metabolites that play a role in biological functions including skeletal homeostasis and repair. AA, a polyunsaturated fatty acid that is an integral part of the cell membrane, is metabolized by Cyclooxygenases (COX) into prostaglandins, prostacyclin, and thromboxane [54]. The unstable prostaglandin intermediates products undergo further transformation by specific prostanoid synthases to form the final products: Prostaglandin E2 (PGE2), Prostaglandin D2 (PGD2), Prostaglandin F2α (pgf 2α), Prostacyclin (pgi2) and Thromboxane (txa2) [55]. pge2 and PGF2α play multiple roles in bone remodeling [56,57].

The multifaceted role of PGE2 signaling in bone remodeling can be attributed through four distinct receptors: EP1R, EP2R, EP3R, and EP4R. Initial research suggested that PGE2 had osteoinductive properties through the EPR receptor and was beneficial in bone repair in mice [58], but others report that PGE2 inhibits BMSC-derived osteoblast matrix mineralization [54]. PGE2 action is primarily mediated through the EP2R and EP4R receptors increasing intracellular Cyclic AMP (cAMP) activity.

PGE2 also plays a role in osteoclasts through EPR4. Choudhary and others reported that PGE2, acting via EP4R on osteoclastic-lineage bone marrow macrophages, grown in the presence of Macrophage-Colony Stimulating Factor (M-CSF), stimulated secretion of a factor or factors that suppressed Parathyroid Hormone (PTH)-stimulated osteoblast differentiation [59]. Initial studies in mice suggested that PGE2 enhanced osteoclastic differentiation in mice [60], but studies conducted with primary human osteoclasts conversely reported that PGE2 strongly inhibits osteoclast formation [61].

PGF2α also regulates multiple bone remodeling activities [57]. PGF2α stimulates sodium-dependent phosphate transport in osteoclasts and upregulates Interleukin-6 (IL-6) synthesis, thus promoting osteocyte-mediated osteoclastogenesis through phosphorylation of Janus kinase 2 (JAK2) and enhanced RANKL secretion [62]. In osteoblasts PGF2α signaling has been shown to promote cell survival through its association with Fibroblast Growth Factor-2 (FGF-2) [57].

The role of prostaglandin receptor signaling in human bone remodeling is not fully understood, but researchers are finding new roles and implications of prostaglandins disease. PGE2 has been implicated in bone disease associated with cystic fibrosis. Genetic mutations associated with the chloride ion channel Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) lead to cystic fibrosis-related osteoporosis. CFTR plays a major role in PGE2 and OPG expression [63]. Prostaglandins have also been implicated in rheumatoid arthritis due to their ability to pathogenically mediate inflammation [64,65].

AA is also metabolized by Lipoxygenase (LOX) to form leukotrienes, inflammatory mediating eicosanoids [66]. LOX catabolizes AA and forms 5-hydroperoxyeicosatetraenoic acid (5-HPETE) which is subsequently converted to leukotriene A4 (LTA4) via leukotriene synthase. LTA4 undergoes additional metabolism by LTA4 hydrolase to form leukotriene B4 (LTB4). LTA4 is also targeted by glutathione S-transferase to produce leukotriene C4 (LTC4) [55].

Like prostaglandins, leukotrienes affect skeletal homeostasis. Current data supports the idea that LTB4 binds its cognate BLT receptors and induces osteoclastogenesis through Phospholipase C (PLC) and calcium signaling culminating in upregulation of osteoclastic genes in precursors [67].

It has been reported that excessive consumption of omega 6 fatty acids, like AA, induce adipogenesis, inhibit osteoblastogenesis, and may contribute to the pathogenesis of obesity and osteoporosis [68]. The immunoregulatory properties of AA metabolites PGE2 and LTB4 have been implicated in Rheumatoid arthritis-associated inflammation and IL-17 secretion [69].

RANK signaling pathway: RANK signaling is a primary regulator of osteoclastogenesis and osteoclast activation and survival. It consists of the RANK receptor found on the plasma membrane of osteoclast precursors and mature osteoclasts, RANKL a receptor-binding ligand secreted by osteoblasts and osteocytes, and Osteoprotegerin (OPG), a soluble molecular mimic of the RANK receptor that sequesters extracellular RANKL, preventing osteoclastic signaling [70].

It has been reported that RANK Ligand (RANKL), a member of the TNF superfamily, secreted by osteocytes initiates trabecular/cancellous bone resorption [71,72], but RANKL secretion has been found in several different cell types including BMSCs, osteoblasts, bone lining cells and lymphocytes [73].

RANK signaling is initiated when RANKL binds RANK and TNF Receptor-Associated Factor (TRAF) proteins are recruited to the RANK cytoplasmic domain. TRAF6 plays a critical signal transduction role because it interacts with several downstream targets including Nuclear Factor-kappa B (NF-κB), c-Jun N-terminal Kinase (JNK), extracellular signal-related kinase (ERK), p38, Akt/Protein kinase B, and Nuclear Factor Of Activated T Cells (NFAT) [74].

The signaling complex formed between RANK and TRAF6 includes TAK1 and TAB and can activate all three MAPK pathways (ERK, JNK, and p38). Downstream signaling through ERK elicits osteoclast proliferation, differentiation, and survival, while JNK and p38 pathways are primarily associated with genes related to osteoclastogenesis, like c-Fos and AP-1 [70]. The RANK-TRAF6 complex also stimulates proto-oncogene tyrosine-protein kinase Src (c-SRC) that activates phosphatidylinositol 3-kinase (PI3K) and Akt/Protein kinase B, a serine/threonine kinase. Akt mediates downstream responses that promote the expression of osteoclastic target genes essential for survival, growth, and proliferation [74].

IκB Kinase 1 (IKK1) also activates the RANK-TRAF6 complex. IKK1 phosphorylates IκBα protein, a kappa B inhibitor that inactivates NF-κB transcription factor blocking nuclear localization signals. Functional NF-κB transcription factor promotes genes that support osteoclast differentiation [75].

Lastly, the activated RANK signaling pathway leads to p38α, an isoform of p38 commonly found at high levels in osteoclast precursors and mature osteoclasts, phosphorylation through TAK1 and MAPK Kinases (MEK) 3 and 6. Activated p38 directly phosphorylates NFATc1 and Miropthalmia-Associated Transcription Factor (MITF), transcription factors essential for osteoclastogenesis [76].

Dysregulation of RANKL can lead to changes in bone homeostasis that lead to disease. For instance, osteoblastic RANKL secretion is influenced directly by estrogen, postmenopausal women with decreased estrogen levels are at risk of excessive osteoclastic RANK signaling and subsequent bone resorption leading to weakened bone density and osteoporosis [77]. Genetic abnormalities in RANK and RANKL genes lead to an absence or greatly reduced bone resorption called autosomal recessive osteopetrosis (ARO) [78]. Aberrant RANK signaling can also be found in osteolytic bone metastasis where the cancer cells produce RANKL eliciting excessive continuous bone resorption [79].

Bone disease and failure

As aforementioned, various cell-signaling pathways regulate bone homeostasis and repair by guiding processes such as inflammation and angiogenesis. Dysregulation of these processes or signaling pathways ultimately lead to disease or bone failure. Understanding the interconnections of these fundamental processes in the normal, inflamed, infected, pharmacologically-manipulated or diseased condition will provide insight into the molecular mechanisms guiding these processes. Our meta-analysis seeks to identify commonalities between bone disease and failure specifically resulting from an imbalance in osteoblast and osteoclast direct bone homeostasis.

Multiple myeloma: Multiple Myeloma (MM) is a hematological cancer of the plasma cell that infiltrates and disrupts normal bone marrow cell function leading to uncoupled bone remodeling. The resulting chronic increase in bone resorption and decreased remodeling promote the formation of osteolytic/osteoclastic lesions as MM progresses to Myeloma Bone Disease (MBD) [80]. In MBD, lesions form as localized discrete lytic lesions, widespread weakened bone tissue/osteopenia, or multiple lytic lesions affecting any part of the skeleton. More than 80% of MM patients suffer from MBD-related bone lesions that are most commonly found in the spine, skull, and long

bones [81]. MM cells express and secrete several different signaling pathway molecules to enhance their proliferation and survival while pathogenically causing lesion formation.

Transformed plasma cells alter normal bone remodeling by disrupting canonical Wnt/β-catenin signaling through the aberrant expression of Lgr4 [82], secretion of Wnt antagonists sclerostin and DKK1 [83], and by stimulating excessive resorption through soluble RANKL secretion [84,85]. The pathogenesis of MBD also involves a negative reciprocal relationship in Notch signaling between MM cells and osteocytes that decreases the number of viable osteocytes in trabecular and cortical bone [86,87].

Blocking Wnt receptors on the cell membrane of osteoblasts through the secretion Wnt inhibitors, sclerostin and DKK1, redirects R-spondins to bind the Lgr4 receptor on the MM cells promoting β-catenin-derived proliferative and survival signaling. MM cells further inhibit osteogenesis and osteoblast differentiation and function through the secretion of soluble RANKL. RANKL binds RANK on osteoclasts activated RANK signaling that promotes osteoclast proliferation and function while initiating caspase-3-mediated apoptosis in osteocytes [88]. MM also quells the TRANCE-regulated secretion of osteoprotegerin, a soluble RANKL decoy receptor, in osteoblasts [89]. Overall, changes in these signaling pathways promote osteoclast function while reducing osteoblast mediate bone regeneration which contributes to the formation of multiple lytic lesions throughout the skeleton.

Diabetes: Diabetes Mellitus (DM) has become one of the leading causes of death in the United States; with the CDC reporting 9.4% of the American populous diagnosed with the disease [90]. Diabetes Mellitus type-1 (DM1) develops from an autoimmune attack on pancreatic β-cells; rendering one incapable of producing insulin. Diabetes Mellitus type-2 (DM2) is identified by the unmediated resistance to insulin and insufficient rate of production [91]. Most commonly identified as a metabolic disease, DM is characterized by increased plasma glucose levels with decreased cellular glucose levels. Disease severity is most commonly associated with comorbidities onset by hyperglycemic-mediated inflammation; such as hypertension, cardiovascular disease, neuropathy, reninopathy, and nephropathy [92]. DM is also culpable for negatively influencing the homeostatic regulation of bone (Figure 1).

The diabetic condition of dysregulated bone homeostasis and subsequent impaired fracture healing is exacerbated by the disjunction in mutualism between bone and glucose homeostasis. Osteoblast differentiation and bone-forming activity is functionally dependent upon cellular uptake of glucose by the Glut1 transporter [93]. Osteoblast differentiation is facilitated by cellular glucose inhibition of 5′ AMP-activated protein Kinase (AMPK) degredation of Runt-related transcription factor 2 (Runx2); therefore, insulin-dependent glucose intolerance from DM impairs osteoblastic differentiation, maturation, and bone formation [93]. In the uncarboxylated form, osteoblast-secreted osteocalcin regulates glucose homeostasis by stimulating pancreatic β-cell proliferation, promoting insulin secretion, and increasing its sensitivity [94]. However, intracellular protein tyrosine phosphatase (OST-PTP) is shown to work upon an unelucidated mechanism which favors the carboxylation of osteoblast-secreted osteocalcin, thereby preventing pro-glucometabolic activity [95]. Decarboxylation of osteoblast-secreted osteocalcin is achieved via osteoclast-acidification of the extracellular matrix; a process mediated by insulin signaling in osteoblasts to favor osteoclast activity and bone resorptive-dependent glucose homeostasis [96]. Increased osteoclast activity during bone resorptive-dependent glucose homeostasis is the result of osteoblast decoy-RANKL receptor osteoprotegerin inhibition, thereby uncoupling osteoblast regulation of osteoclastogenic NF-kB/RANKL activation [97].

Downregulated osteoblast activity is additionally propogated by DM related hyperglycemia, resulting in elevated Advanced Glycation Endproducts (AGEs) that promote osteocyte secretion of sclerostin [98]. Increased serum levels of osteocyte-secreted sclerostin inhibit osteoblast differentiation and bone forming activity by antagonistic supression of the Wnt/β-catenin signaling pathway via lipoprotein receptor-related protein 5 or 6 (LPR5/6) binding [99]. AGE induced bone resorption is also achieved by increasing RANKL expression through AGE Receptor (RAGE) activation of transcription factor NF-κB, and by RAGE inhibition of osteoprotegerin expression [100].

In the diabetic condition, it is proposed that the synergetic relationship between bone and glucose homeostasis is stressed by the heightened demand for insulin-mediated glucose transport, resulting in prolonged states of bone resorption to mediate increased plasma glucose levels. In comparison to non-diabetic controls, diabetic fracture healing operates at a delayed rate with reduced efficacy in restoring the structural integrity and strength of bone; featured by the gradual recondition of bone density [101], and the latent reclaimation of tensile and mechanical strength [102]. The declined rate of diabetic fracture healing and bone turnover is consequent of dysregulated homeostatic control of coupled osteoblast and osteoclast activity; attenuated by insulin signaling in osteoblasts, hyperglycemic production of Advanged Glycation Endproducts (AGEs), and inhibition of osteogenic progenitor stem cell differentiation.

Rosiglitazone is an antidiabetic drug that acts to increase insulin sensitivity, thereby lowering plasma glucose levels [103]. Rosiglitazone is an agonist for the peroxisome Proliferator-Activated Receptor Gamma (PPARγ). Insulin sensitivity is strengthened as a result of PPARγ activated signaling pathways; however, rosiglitazone is also responsible for bone loss [104]. Rosiglitazone activated PPARγ signaling has been shown increase Prolyl Hydroxylase Domain Protein (PDH) expression, resulting in the downregulation of runt-related transcription factor-2 (Runx2), thereby inhibiting osteoblast differentiation. Additionally, PPARγ signally stimulates osteoclast differentiation from Hematopoietic stem cells, resulting in larger decoupling between osteoclast and osteoblast coupling [105]. In this way, rosiglitazone is liable for deficient bone remodeling and progressive bone loss [106].

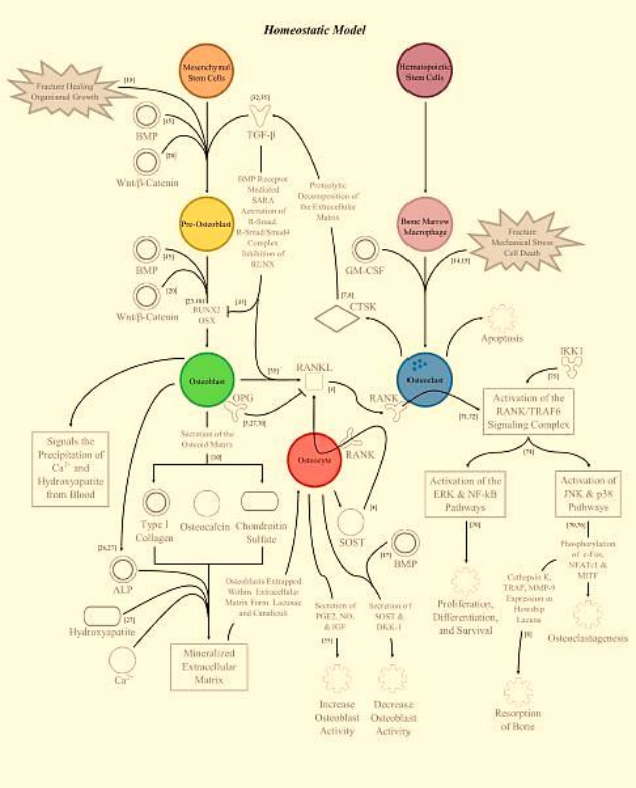

Figure 1: Homeostatic Model of Osteoblast and Osteoclast Coupling. This figure illustrates the major pathways and cell signaling mediators involved in the homeostatic signaling which drives normal bone remodeling. The complex and regulated communication pathways which dictate physiological responses to a dynamic bone-microenvironment are detailed in the current review; however, for more information on a specific topic, please refer to the corresponding references adjacent to the respective pathway or signaling mediator within the figure.

Rheumatoid arthritis: Rheumatoid Arthritis (RA) is an autoimmune disease characterized by systemic chronic inflammation of the joints, resulting in the progressive damage of cartilage and juxta-articular bone, affecting nearly 1% of the world population [107]. RA elicits an autoimmune attack on the joint tissues, resulting in prolonged inflammation and local pro-inflammatory cell signaling. The synovial tissue lining the interior of the joint inflames, preventing the secretion of lubrication for smooth joint movement. Unmediated inflammation of joint tissues can result in irreversible deterioration of joint cartilage and connected bone. Those suffering from RA experience chronic pain, joint immobility, and peripheral swelling; with many relying on disability to perform necessary tasks. Bone loss attributed to RA perpetuates disease symptoms; however, the systemic signaling of the arthritic immune response effects other orthopedic activity, suggesting a unique regulatory relationship between RA and normal bone homeostatic mechanisms.

Arthritic inflammation of the synovial tissue is associated with activated signaling pathways that favor excessive resorption of the surrounding bone and the dysregulation of bone homeostasis [108]. Synovial fibroblast expression of RANKL is unique to the arthritic condition and is elevated such that osteoclast differentiation and resorptive activity is disproportionately increased in comparison to osteoblast activity [109]. Additionally, the osteoprotegerin regulatory mechanism over RANKL osteoclast activation is downregulated by limited competitive inhibition relative to the overexpression of RANKL [110]. Failure to remodel bone at sites of arthritic erosion is supported by the suppression of osteoblast activity. Another mechanism of osteoblast inhibition is founded in the elevated expression of Dikkopf proteins (DKK) in arthritic synovial tissues, a known antagonist of the Wnt/ β-catenin pathway responsible for upregulating osteoblast differentiation and the maturation of functional osteoblasts [111]. Arthritic inflammation is also responsible for the upregulated synovial expression of TNF-α, a pro-inflammatory cytokine that promotes M-CSF mediated activation of osteoclast differentiation [112]. Additionally, the absence of osteoblast remodeling at the sites of erosion can be attributed to TNF-α induced apoptosis of osteoblasts, further illustrating dysregulated homeostasis of arthritic bone [113].

Osteoporosis: Osteoporosis is a bone degenerative disease, characterized by low bone density; leading to bone fragility and subsequent risk to fracture [114]. MSC differentiation, extracellular matrix formation, angiogenesis, and callus remodeling are all orchestrated by complex signaling pathways. Dysregulation of these pathways can result in the osteoporotic condition. Pathogenesis of osteoporosis has multiple derivations; age, sex, genetic predisposition, variable hormone signaling, and nutritional deficiencies all contributing towards disease onset.

While osteoporosis can affect both men and women of all ages, there is a significant increase in prevalence within the subset of postmenopausal females [115]. Estrogen directly downregulates osteoclast-dependent bone resorption by inhibition of osteoclastogenesis [116]. Postmenopausal females are therefore prone to dysregulated bone homeostasis from inadequate osteoclast suppression. Inhibition of estrogen signaling is shown to directly increase expression of interleukin-6 (IL-6), a pro-inflammatory cytokine which elevates osteoclast differentiation and activity through increased RANKL signaling [117,118]. Furthermore, after menopause, estrogen-mediated bone remodeling becomes dysregulated, favoring the differentiation of osteoclasts; thereby increasing their resorptive activity and ultimately resulting in reduced bone mineral density [119].

Glucocorticoids (GCs) have been prescribed to treat pain associated with chronic inflammation. While GCs are beneficial for mediating short-term chronic pain, some depend on long-term use to treat chronic inflammatory disease-related pain. Extended GC use has a significant deleterious impact on bone physiology; preventing fracture healing and causes further osseous deterioration. Long-term use of GCs can decrease osteoblast activity by inducing osteoblastic apoptosis [120]; thereby impeding restorative bone-modeling activity [121]. Likewise, the downstream signaling from osteoblasts for skeletal homeostatic regulation is compromised, further perpetuating dysregulated bone metabolism. Long-term GC use is associated with lowered incidence of fracture healing [122], as well as

attenuating bone-related diseases such as rheumatoid arthritis and osteoporosis [123].

Steroidal osteoporosis has become the second most common cause of osteoporotic fracture and decreased bone density. It occurs after steroidal use inhibits osteoblast differentiation [124] and suppresses Wnt/β-catenin pathway signaling [125]. Suppression of Wnt/β-catenin signaling can result from a glucocorticoid-induced elevation of secreted frizzled-related protein 1 (SFRP1), a known antagonist of the Wnt/β-catenin pathway towards osteoblast differentiation [126]. Steroidal effects on osteoblast signaling show decreased expression of osteoprotegerin as well as elevated expression of RANKL, further demonstrating that steroidal use can lead to dysregulated bone resorption [127].

Craniometaphyseal dysplasia: Craniometaphyseal Dysplasia (CMD) is a hereditary bone disease resulting in craniofacial bone malformation, gradual nerve damage, and long bone distortion. If left untreated, the progression of CMD can cause blindness, deafness, and facial palsy [128]. CMD causes excessive bone mineralization resulting from decreased pyrophosphate (PPi) activity, an identified mineralization inhibitor [129]. Progressive ankylosis protein (ANK) mutation, in the case of dominant CMD, is unable to transport PPi to the extracellular fluid, thereby supporting excessive mineralization [130].

Inhibition of ANK-PPi transport increases bone density by preventing hydroxyapatite deposition. PPi is hydrolyzed to inorganic phosphate (Pi) via alkaline phosphatase (ALP), affixing to calcium to form hydroxyapatite. In some cases, unregulated mineralization presented conditions of hypocalcemia, with upregulated ALP and parathyroid hormone (PTH) levels [129]. PTH has been shown to stimulate bone resorption to release calcium, restoring calcium homeostasis in CMD attenuated hypocalcemia [129]. Increased levels of extracellular ALP and calcium allow for elevated hydroxyapatite deposition. The increased mineralization of bone tissue thereby perpetuates the symptomatic progression of CMD [129].

A second study on CMD's effects on ANK mutations reported decreased osteoclast function facilitating bone resorption, thereby propagating abnormal bone growth in CMD patients [131]. The resorptive function of osteoclasts is predicated upon its ability to adhere to the bone surface and form a tight seal. Mutation of the ANK protein is associated with the production of smaller osteoclasts with improperly formed actin rings, resulting in a disrupted seal which hinders the resorption of bone and results in excessive bone formation [132].

Osteopetrosis: Osteopetrosis (OP) is a genetically derived bone disease characterized by increased bone density; having three heritable patterns: autosomal dominant (ADO), autosomal recessive (ARO), and X-linked (XLO) [133]. OP can be caused by one of 10 known mutations, resulting in failed osteoclast maturation, allowing for unregulated bone growth [134]. Variability in osteoclast differentiation and activity implicate failure of bone resorption, and with a multitude of gene mutations attributing to OP, symptom severity exists on a spectrum. The most common form of OP arises from an autosomal dominant mutation of the chlorine channel 7 (ClC-7) gene, resulting in upregulated osteoclast differentiation that is ultimately nonfunctional [135]. Increased intracellular chloride activates the phosphorylation of Rac1/Cdc42, a small GTPase that activates microphthalmia-associated transcription factor (MITF) and RANK; this activation induces osteoclast differentiation but is associated with failed membrane polarization resulting in loss of function [135].

Viral and bacterial infection: Various viral infections are known to contribute to bone homeostasis dysregulation by altering canonical pathway signaling including human immunodeficiency virus (HIV), hepatitis C, arthritogenic alphaviruses, and Pneumovirus (PVM). HIV has been shown to promote bone loss, associated with the increased RANKL signaling promotion of resorption [136]. HIV infection of T-cells has been shown to augment B-cell expression osteoprotegerin towards RANKL expression through dysregulated immune signaling [137]. The altered condition results in a systemic pro-osteoclastic activity which can lead to excessive bone loss. Viral proteins are also identified as regulators of bone homeostatic mechanisms. HIV proteins Nef and Tat can downregulate the differentiation of mesenchymal stem cells into osteoblasts via NF-kB activated senescence and can upregulate osteoclastogenesis by increasing TNF-α expression in precursor cells [138]. Additionally, Nef, is associated with enhancing osteoclast actin organization of podosomes, cell migration, and fusion such that the osteolytic activity of resorption is increased [139]. Hepatitis C (HpC) is widely characterized by cirrhosis of the liver, symptomatic of hyperbilirubinemia. Bilirubin is associated with bone loss through inhibition of osteoblast differentiation by direct suppression of Runx2 expression [140]. Chikungunya virus (CHIKV), o'nyongnyong virus (ONNV), Ross River virus (RRV), Barmah Forest Virus (BFV), and Sindbis virus (SINV) are a group of arthritogenic alphaviruses associated with impaired bone health. RRV infection of human osteoblasts has been shown to induce osteoclastogenesis via elevated IL-6 expression and RANKL signaling, thereby compelling bone loss [141]. Similar pathogenesis of CHIKV suggests a commonality between elevated pro-inflammatory cytokine expression and other arthritogenic alphaviruses [142]. Pneumovirus (PVM) infection is reported to induce osteoblast apoptosis, associated with increased TNF-α expression. Additionally, decreased osteocalcin expression supports impaired bone growth. Furthermore, severe inflammation from acute viral infection has been shown to promote osteoclastogenesis contributing to impaired bone growth [143].

Staphylococcus aureus (*S. aureus*) is a bacterial agent notable for the onset of osteomyelitis. S. aureus infection of osteoblasts provokes the release of cytokines responsible for stimulating the immune response as well as promoting osteoblast apoptosis and osteoclastogenesis, thereby causing extensive bone loss in affected tissues [144]. Bacterial infection of Aggregatibacter actinomycetemcomitans has been shown to inhibit bone growth by simulating osteoblastic apoptosis via caspase-3 signaling [145]. Overall, viral and bacterial infections tend to stimulate osteoclastogenic behavior and result in reduced bone formation (Figure 2).

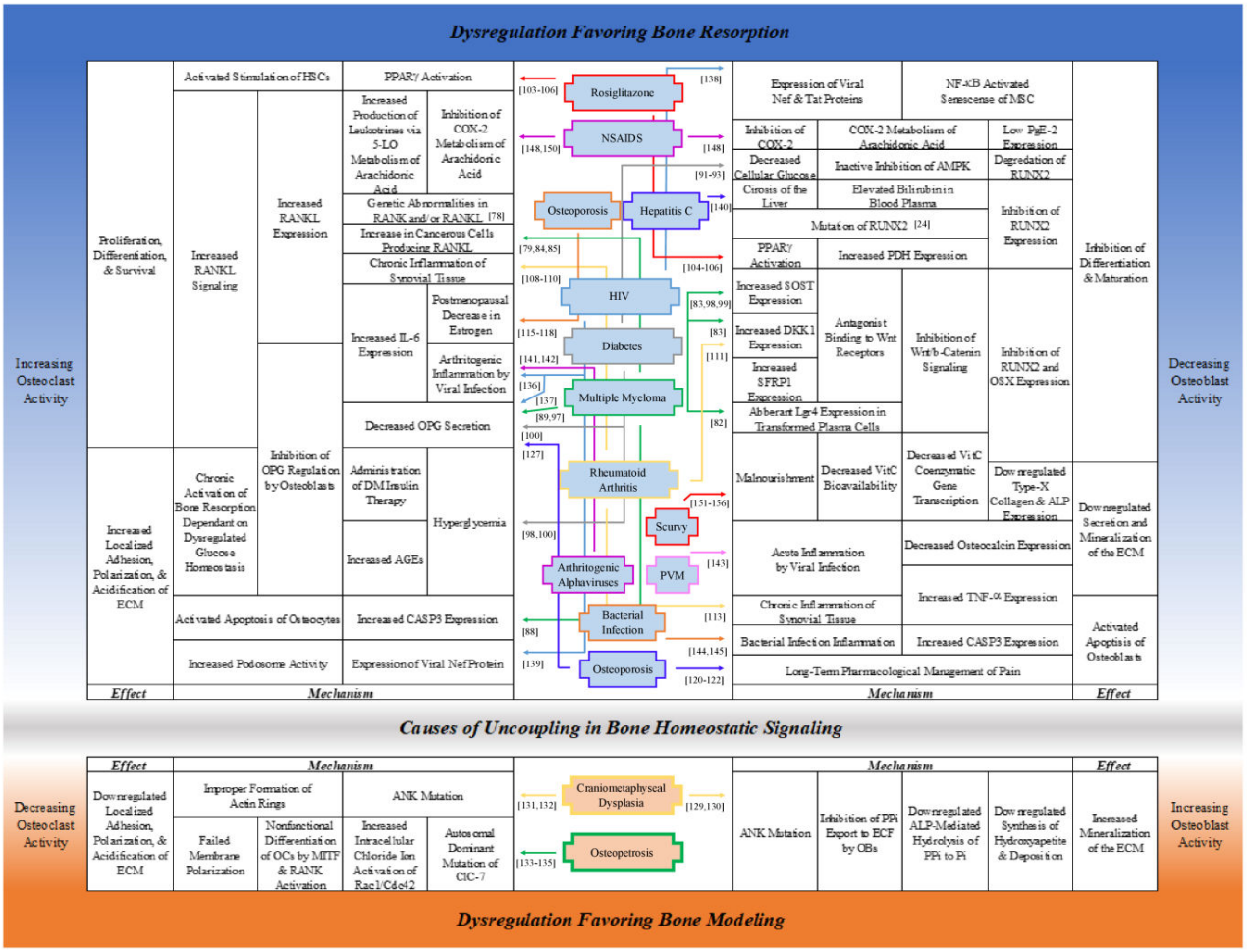

Figure 2: Summary of Key Mechanisms Involved in the Disruption of Bone Remodeling and Homeostatic Signaling. This figure simplifies mechanisms which describe how various topics compromise the coupling between osteoblasts and osteoclasts in normal bone remodeling and homeostatic signaling. The central column of the figure contains fifteen various topics that are implicated in the disruption of bone homeostasis. To understand the mechanism by which a certain topic disrupts bone remodeling and homeostatic signaling, follow the color coordinated arrow(s) leaving the central topic to the box at which the arrow terminates. This box, whether it be on the right or left side of the central column, is the first major factor involved in the mechanism. If, for example, the first box is on the right side of the central column, the box, or boxes, in the next rightward column that touch the first box is/are the next sequential component(s) of the mechanism. Similarly, if the first box is on the left side of the central column, the box, or boxes, in the next leftward column that touch the first box is/are the next sequential component(s) of that mechanism. Mechanisms with a rightward progression ultimately dysregulate bone homeostasis by altering osteoblast dynamics; whereas mechanisms with a leftward progression ultimately alter osteoclast dynamics. In the context of dysregulating homeostatically coupled osteoblast-osteoclast activities; mechanisms terminating within the upper blue region of the figure drive dysregulation towards bone resorption, achieved by decreasing osteoblast activity (rightward mechanisms) and/or increasing osteoclast activity (leftward mechanisms). Likewise, mechanisms terminating within the lower orange region of the figure drive dysregulation towards bone modeling, achieved by increasing osteoblast activity (rightward mechanisms) and/or decreasing osteoclast activity (leftward mechanisms). Therefore, topics located within the blue hemisphere of the figure are known to uncouple bone homeostasis favoring abnormal bone loss, and those in the orange hemisphere favoring abnormal growth. For more information detailing the known components of a mechanism, please refer to the corresponding references identified immediately below the terminating end of each respective arrow.

Other notable bone homeostasis modulators

Bisphosphonates: Bisphosphonate has been medicinally used to treat metabolic bone diseases such as osteoporosis, boasting a decreased rate in bone fracture. However, time-spanning high-dose bisphosphonate therapies among oncology patients have been associated with osteonecrosis of the jaw (ONJ) [146]. The bisphosphonate-ONJ relationship holds a low rate of incidence and is yet to be understood by a definitive mechanism. Likewise, it is hypothesized that bisphosphonate related ONJ is the result of multiple factors that have a combined effect on ONJ onset.

Osteonecrosis is associated with low rates of bone turnover; a characteristic related to bisphosphonate's inhibition of osteoclast activity [146]. Subsequently, ONJ may be rooted in an osseous infection, unable to be readily removed due to bisphosphonate inhibition of resorptive cells [147]. The mechanism for how bisphosphonate dysregulates bone

homeostasis is not well understood, and more research needs to be done to understand its mode of action.

NSAIDs: When individuals fracture a bone, they typically suffer from pain as a result of the trauma. Historically, nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin, Motrin, or Advil were taken as over the counter pain killers to mediate the fracture-associated pain. It was observed that those who took such NSAIDs post-fracture had a significantly lower rate of healing [17].

The relationship between NSAIDs and reduced bone formation is considered to be associated with NSAID inhibition of cyclooxygenase-2 (COX2) enzymes. COX2 metabolizes arachidonic acid to synthesize prostaglandins associated with mediating pain and inflammation; however, prostaglandin mediation of osteoblast and osteoclast activity is the proposed target of NSAID related bone dysregulation [148]. Differentiation of mesenchymal stem cells into osteoblasts is regulated by the expression of BMP-2 and PgE2, both of which are shown to be downregulated in the condition of NSAID treatment [149]. Additionally, it is considered that NSAID inhibition of COX2 metabolism ushers arachidonic acid towards 5-lipoxygenase (5-LO) metabolism, producing leukotrines responsible for stimulating osteoclast activity while downregulating osteoblast activity [150]. NSAID inhibition of the COX2 metabolic pathway of arachidonic acid is shown to dysregulate mechanisms of osteogenic homeostasis, therefore alternative pain management medication is suggested for prolonged use.

Scurvy: Scurvy, a nautically-rooted malnourishment disease, prevails in those deficient in ascorbic acid ($C_6H_8O_6$). Ascorbic acid, commonly referred to as vitamin C (Vit C), is a water-soluble essential vitamin, metabolically sourced from the consumption of green vegetables and citrus fruits. Alcoholism, drug abuse, mental illness, and poverty are all risk factors for disease; however, the provenance of scurvy is malnourishment [151]. Lack of proper nutrition results in vitamin C deficiency; thus, launching a systematic cessation of activity within cellularly-dependent mechanisms. The disease manifests in individuals with a reduced daily vitamin C intake of less than 75-90 mg over 8-12 weeks, with symptoms increasing in severity when left untreated [152]. Léger details a range of symptoms inclusive of anemia, hemolysis, edema, bone pain, bruising, gum disease, jaundice, fever, improper wound healing, seizure, depression, neuropathy, and in late-chronic stages death [152].

Vitamin C deficiency is associated with decreased stimulation of osteoblast differentiation and activated osteoclastogenesis [153]. VitC aids in osteoblast differentiation by acting as a cofactor for transcriptional activation of gene products, such as osterix, that are required for differentiation [154]. Here, it is observed that VitC does not act solely on genetic expression of osteogenic cells, but rather acts as a cofactor for transcriptional regulation of genes required to facilitate bone homeostasis. Additionally, bioactive Vit C functions co-enzymatically and as a reducing agent within mechanisms essential for bone and connective tissue maintenance. These metabolic pathways are notable for collagen development, the stratal fundament to bone; as well as capillary and connective tissue formation [155].

A major phase of endochondral ossification involves chondrocyte hypertrophy; producing calcified cartilage from the deposition of its hardened extracellular matrix post apoptosis. It has been shown that Vit C is essential for regulating hypertrophic chondrocyte gene expression responsible for initiating matrix calcification. Hypertrophic chondrocytes are reliant on the presence of Vit C to induce the upregulation of type X collagen expression and the activation of alkaline phosphatase. This increase in activity has been shown to stimulate matrix mineralization. The absence of Vit C in the chronic state can compromise collagen synthesis, resulting in consequent downstream effects in the homeostasis of bone.

CONCLUSION

Bone remodeling is an integral part of counteracting age-associated bone tissue decomposition and in healing aberrant bone fractures, but there are several heritable and acquired bone disorders that dysregulate and uncouple bone formation and resorption. The pathophysiology of many diseases that affect bone health is attributed to the disruption of osteogenic molecular signaling pathways that ultimately control cellular differentiation, proliferation, survival, functionality, and localization. Inter and intracellular signaling events direct transcriptional regulation of osteometric genes and their concordant proteins and provide essential cues that control the transition through the different phases of the bone remodeling process. The complex interconnectivity between receptor activation or inhibition, secretion or sequestration of molecular signaling moieties, and the cellular microenvironment that directs cell fate is not fully understood, but the roles of several well-defined pathways attributed to a number of bone disorders have been identified. This review explored the causal association between several well-recognized conditions and the mechanistic properties that alter the effectiveness of the bone remodeling process.

REFERENCES

1. Frost HM. Tetracycline-based histological analysis of bone remodeling. Calcif Tissue Res. 1969;3:211-237.
2. Eriksen EF. Cellular mechanisms of bone remodeling. Rev Endocr Metab Disord. 2010;11:219-227.
3. The Basics of Bone in Health and Disease. 2004.
4. Compton JT, Lee FY. A review of osteocyte function and the emerging importance of sclerostin. J Bone Joint Surg Am. 2014;96:1659-1668.
5. Boyce BF, Xing L. Functions of RANKL/RANK/OPG in bone modeling and remodeling. Arch Biochem Biophys. 2008;473:139-146.
6. Ginaldi L, Martinis MD. Osteoimmunology and beyond. Curr Med Chem. 2016;23:3754-3774.
7. Delaisse JM. The reversal phase of the bone-remodeling cycle: cellular prerequisites for coupling resorption and formation. Bone Key Rep. 2014;3:561.
8. Dodds RA, James IE, Rieman D, Ahern R, Hwang SM, Connor JR, et al. Human osteoclast cathepsin K is processed intracellularly prior to attachment and bone resorption. J Bone Miner Res. 2001;16:478-486.
9. Torres AYP, Flores MV, Orozco L, Cruz RV. Molecular aspects of bone remodeling. Osteoporosis. 2013.

10. Florencio SR. Biology of bone tissue: structure, function, and factors that influence bone cells. Biomed Res Int. 2015:421746.
11. Recker RR. Safety of bisphosphonates in the treatment of osteoporosis. Am J Med. 2009;122:22-32.
12. Buenzli PR, Sims NA. Quantifying the osteocyte network in the human skeleton. Bone. 2015;75:144-150.
13. Manolagas SC. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr Rev. 2000;21:115-137.
14. Raggatt LJ, Partridge NC. Cellular and molecular mechanisms of bone remodeling. J Biol Chem. 2010;285:25103-25108.
15. Feng X, McDonald JM. Disorders of bone remodeling. Annu Rev Pathol. 2011;6:121-145.
16. Solheim E. Growth factors in bone. Int Ortho. 1998;22:410-416.
17. Oconnor J, Lysz T. Celecoxib, NSAIDs and the skeleton Drugs Today. 2008;44:693.
18. Akkiraju H. Nohe A. Role of chondrocytes in cartilage formation, progression of osteoarthritis and cartilage regeneration. J Dev Biol. 2015;3:177-192.
19. Steinhart Z, Angers S. Wnt signaling in development and tissue homeostasis. Dev. 2018;145.
20. Liu AR. Activation of canonical wnt pathway promotes differentiation of mouse bone marrow-derived MSCs into type II alveolar epithelial cells, confers resistance to oxidative stress, and promotes their migration to injured lung tissue in vitro. J Cell Physiol. 2013;228:1270-1283.
21. Stamos JL, Weis WI. The β-Catenin destruction complex. Cold Spring Harb Perspect Biol. 2013.
22. Mukherjee A. Understanding How Wnt influences destruction complex activity and beta-catenin dynamics. ISCI. 2018;6:13-21.
23. Gaur T. Canonical WNT signaling promotes osteogenesis by directly stimulating Runx2 gene expression. J Biol Chem. 2005;280:33132-33140.
24. Lee KE. RUNX2 mutations in cleidocranial dysplasia. Genet Mol Res. 2013;12:4567-4574.
25. Salazar VS. Specification of osteoblast cell fate by canonical Wnt signaling requires Bmp2. Dev. 2016;143:4352-4367.
26. Rawadi G. BMP-2 controls alkaline phosphatase expression and osteoblast mineralization by a Wnt autocrine loop. J Bone Miner Res. 2003;18:1842-1853.
27. Halling LC. Bone alkaline phosphatase and tartrate-resistant acid phosphatase: Potential co-regulators of bone mineralization. Calcif Tissue Int. 2017;101:92-101.
28. Chen H, Li J, Wang Q. Associations between bone-alkaline phosphatase and bone mineral density in adults with and without diabetes. Med. 2018;97:0432.
29. Regard JB. Wnt signaling in bone development and disease: making stronger bone with Wnts. Cold Spring Harb Perspect Biol. 2012;4.
30. Schneider VA, Mercola M. Wnt antagonism initiates cardiogenesis in Xenopus laevis. Genes Dev. 2001;15:304-315.
31. Wu M, Chen G, Li YP. TGF-β and BMP signaling in osteoblast, skeletal development, and bone formation, homeostasis and disease. Bone Res. 2016;4:16009.
32. Yasui T. Regulation of RANKL-induced osteoclastogenesis by TGF-beta through molecular interaction between Smad3 and Traf6. J Bone Miner Res. 2011;26:1447-1456.
33. Shi M. Latent TGF-β structure and activation. Nature. 2011;474:343-349.
34. Munger JS. Sheppard D, Cross talk among TGF-β signaling pathways, integrins, and the extracellular matrix. Cold Spring Harb Perspect Biol. 2011;3:005017.
35. Franceschi RT, Ge C. Control of the osteoblast lineage by mitogen-activated protein kinase signaling. Curr Mol Biol Rep. 2017;3:122-132.
36. Guzman A. SMAD versus non-SMAD signaling is determined by lateral mobility of bone morphogenetic protein (BMP) receptors. J Biol Chem. 2012;287:39492-39504.
37. Rahman MS. TGF-β/BMP signaling and other molecular events: regulation of osteoblastogenesis and bone formation. Bone Res. 2015;3:15005.
38. Lu G. TAB-1 modulates intracellular localization of p38 MAP kinase and downstream signaling. J Biol Chem. 2006;281:6087-6095.
39. Greenblatt MB. The p38 MAPK pathway is essential for skeletogenesis and bone homeostasis in mice. J Clin Invest. 2010;120:2457-2473.
40. Sarahrudi K. Elevated transforming growth factor-beta 1 (TGF-beta1) levels in human fracture healing. Injury. 2011;42:833-837.
41. Ehnert S. TGF-beta1 as possible link between loss of bone mineral density and chronic inflammation. PLoS One. 2010;5:14073.
42. Robertson IB, Rifkin DB. Regulation of the bioavailability of TGF-β and TGF-β-related proteins. Cold Spring Harb Perspect Biol. 2016.
43. Grafe I. Excessive transforming growth factor-beta signaling is a common mechanism in osteogenesis imperfecta. Nat Med. 2014;20:670-675.
44. Sun J. The transforming growth factor-beta1 (TGF-beta1) gene polymorphisms (TGF-beta1 T869C and TGF-beta1 T29C) and susceptibility to postmenopausal osteoporosis: a meta-analysis. Med. 2015;94:461.
45. Garimella R. Expression and synthesis of bone morphogenetic proteins by osteoclasts: a possible path to anabolic bone remodeling. J Histochem Cytochem. 2008;56:569-577.
46. Wang RN. Bone morphogenetic protein (BMP) signaling in development and human diseases. Genes Dis. 2014;1:87-105.
47. Chen G, Deng C, Li YP. TGF-beta and BMP signaling in osteoblast differentiation and bone formation. Int J Biol Sci. 2012;8:272-288.
48. Miyazono K, Kamiya Y, Morikawa M. Bone morphogenetic protein receptors and signal transduction. J Biochem. 2010;147:35-51.
49. Dudaric L. Expression of the BMP-2, -4 and -7 and their antagonists gremlin, chordin, noggin and follistatin during ectopic osteogenesis. Coll Antropol. 2013;37:1291-1298.
50. Krause C, Guzman A, Knaus P. Noggin. Int J Biochem Cell Biol. 2011;43:478-481.
51. Billings PC. Dysregulated BMP signaling and enhanced osteogenic differentiation of connective tissue progenitor cells from patients with fibrodysplasia ossificans progressiva (FOP). J Bone Miner Res. 2008;23:305-313.
52. Liu DB. Association of bone morphogenetic protein (bmp)/smad signaling pathway with fracture healing and osteogenic ability in senile osteoporotic fracture in humans and rats. Med Sci Monit. 2018;24:4363-4371.
53. Luo Y. Decreased circulating BMP-9 levels in patients with Type 2 diabetes is a signature of insulin resistance. Clin Sci. 2017;131:239-246.
54. Mirsaidi A, Tiaden AN, Richards PJ. Prostaglandin E2 inhibits matrix mineralization by human bone marrow stromal cell-derived osteoblasts via Epac-dependent cAMP signaling. Sci Rep. 2017;7:2243.
55. Hanna VS, Hafez EAA. Synopsis of arachidonic acid metabolism: A review. J Adv Res. 2018;11:23-32.

56. Raisz LG. Physiologic and pathologic roles of prostaglandins and other eicosanoids in bone metabolism. J Nutr. 1995;125:2024S-2027S.
57. Agas D. Prostaglandin F2alpha: a bone remodeling mediator. J Cell Physiol. 2013;228:25-29.
58. Tanaka M. Prostaglandin E2 receptor (EP4) selective agonist (ONO-4819.CD) accelerates bone repair of femoral cortex after drill-hole injury associated with local upregulation of bone turnover in mature rats. Bone. 2004;34:940-948.
59. Choudhary S. Prostaglandin E2 acts via bone marrow macrophages to block PTH-stimulated osteoblast differentiation in vitro. Bone. 2013;56:31-41.
60. Kobayashi Y. Prostaglandin E2 enhances osteoclastic differentiation of precursor cells through protein kinase A-dependent phosphorylation of TAK1. J Biol Chem. 2005;280:11395-11403.
61. Take I. Prostaglandin E2 strongly inhibits human osteoclast formation. Endocrinol. 2005;146:5204-5214.
62. Wu Q. IL-6 enhances osteocyte-mediated osteoclastogenesis by promoting JAK2 and RANKL activity in vitro. Cell Physiol Biochem. 2017;41:1360-1369.
63. Jacquot J. Bone disease in cystic fibrosis: new pathogenic insights opening novel therapies. Osteoporos Int. 2016;27:1401-1412.
64. Gheorghe KR. Prostaglandin E2 synthesizing enzymes in rheumatoid arthritis B cells and the effects of B cell depleting therapy on enzyme expression. PLoS One. 2011;6:16378.
65. Fattahi MJ, Mirshafiey A. Prostaglandins and rheumatoid arthritis. Arthritis. 2012:239310.
66. Sorgi CA. Dormant 5-lipoxygenase in inflammatory macrophages is triggered by exogenous arachidonic acid. Sci Rep. 2017;7:10981.
67. Dixit N. Leukotriene B4 activates intracellular calcium and augments human osteoclastogenesis. Arthritis Res Ther. 2014;16:496.
68. Casado DA. The omega-6 arachidonic fatty acid, but not the omega-3 fatty acids, inhibits osteoblastogenesis and induces adipogenesis of human mesenchymal stem cells: potential implication in osteoporosis. Osteoporos Int. 2013;24:1647-1661.
69. Chen H. Effects of leukotriene B4 and prostaglandin E2 on the differentiation of murine Foxp3+ T regulatory cells and Th17 cells. Prostaglandins Leukot Essent Fatty Acids. 2009;80:195-200.
70. Walsh MC, Choi Y. Biology of the RANKL-RANK-OPG system in immunity, bone, and beyond. Front Immunol. 2014;5:511.
71. Xiong J, Obrien CA. Osteocyte RANKL: new insights into the control of bone remodeling. J Bone Miner Res. 2012;27:499-505.
72. Xiong J. Osteocyte-derived RANKL is a critical mediator of the increased bone resorption caused by dietary calcium deficiency. Bone. 2014;66:146-154.
73. Feng X, Teitelbaum SL. Osteoclasts: New Insights. Bone Res. 2013;1:11-26.
74. Kim JH, Kim N. Signaling pathways in osteoclast differentiation. Chonnam Med J. 2016;52:12-17.
75. Lamothe B. Site-specific Lys-63-linked tumor necrosis factor receptor-associated factor 6 auto-ubiquitination is a critical determinant of I kappa B kinase activation. J Biol Chem. 2007;282:4102-4112.
76. Cong Q. p38alpha MAPK regulates proliferation and differentiation of osteoclast progenitors and bone remodeling in an aging-dependent manner. Sci Rep. 2017;7:45964.
77. Streicher C. Estrogen regulates bone turnover by targeting rankl expression in bone lining cells. Sci Rep. 2017;7:6460.
78. Pangrazio A. RANK dependent autosomal recessive osteopetrosis: characterization of five new cases with novel mutations. J Bone Miner Res. 2012;27:342-351.
79. Sisay M, Mengistu G, Edessa D. The RANK/RANKL/OPG system in tumorigenesis and metastasis of cancer stem cell: potential targets for anticancer therapy. Onco Targets Ther. 2017;10:3801-3810.
80. Kumar SK. Multiple myeloma. Nat Rev Dis Primers. 2017;3:17046.
81. Hameed A. Bone disease in multiple myeloma: pathophysiology and management. Cancer Growth Metastasis. 2014;7:33-42.
82. Andel VH. Aberrantly expressed LGR4 empowers Wnt signaling in multiple myeloma by hijacking osteoblast-derived R-spondins. Proc Natl Acad Sci. 2017;114:376-381.
83. McDonald MM. Inhibiting the osteocyte-specific protein sclerostin increases bone mass and fracture resistance in multiple myeloma. Blood. 2017;129:3452-3464.
84. Jakob C. Serum levels of total-RANKL in multiple myeloma. Clin Lymphoma Myeloma. 2009;9:430-435.
85. Buckle CH. Soluble rank ligand produced by myeloma cells causes generalised bone loss in multiple myeloma. PLoS One. 2012;7:41127.
86. Delgado CJ. Bidirectional notch signaling and osteocyte-derived factors in the bone marrow microenvironment promote tumor cell proliferation and bone destruction in multiple myeloma. Cancer Res. 2016;76:1089-1100.
87. Terpos E. Pathogenesis of bone disease in multiple myeloma: from bench to bedside. Blood Cancer J. 2018;8:7.
88. Kennedy OD. Osteocyte apoptosis is required for production of osteoclastogenic signals following bone fatigue in vivo. Bone. 2014;64:132-137.
89. Pearse RN. Multiple myeloma disrupts the TRANCE/osteoprotegerin cytokine axis to trigger bone destruction and promote tumor progression. Proc Natl Acad Sci. 2001;98:11581-11586.
90. CDC Press Releases. CDC. 2016.
91. Olokoba AB, Obateru OA, Olokoba LB. Type 2 diabetes mellitus: A review of current trends. Oman Med J. 2012;27:269-273.
92. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. J Clin Investigation. 2005;115:1111-1119.
93. Wei J. Glucose uptake and Runx2 synergize to orchestrate osteoblast differentiation and bone formation. Cell. 2015;161:1576-1591.
94. Lee NK. Endocrine regulation of energy metabolism by the skeleton. Cell. 2007;130:456-469.
95. Rached MT. FoxO1 expression in osteoblasts regulates glucose homeostasis through regulation of osteocalcin in mice. J Clin Investigation. 2010;120:357-368.
96. Ferron M. Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. Cell. 2010;142:296-308.
97. Lecka CB. Diabetes, bone and glucose-lowering agents: basic biology. Diabetologia. 2017;60:1163-1169.
98. Sellmeyer DE. Skeletal metabolism, fracture risk, and fracture outcomes in type 1 and type 2 diabetes. Diabetes. 2016;65:1757-1766.
99. Sanches CP, Vianna AGD, Barreto FDC. The impact of type 2 diabetes on bone metabolism. Diabetol Metab Syndr. 2017;9:85.
100. Jiao H, Xiao E, Graves DT. Diabetes and its effect on bone and fracture healing. Current Osteoporosis Reports. 2015;13:327-335.
101. Sundararaghavan V. Diabetes and bone health: latest evidence and clinical implications. Ther Adv in Musculoskel Disease. 2017;9:67-74.

102. Funk JR. Biomechanical evaluation of early fracture healing in normal and diabetic rats. J Ortho Res. 2000;18:126-132.

103. https://pubchem.ncbi.nlm.nih.gov/compound/Rosiglitazone

104. Broulik PD, Sefc L, Haluzik M. Effect of PPAR-gamma agonist rosiglitazone on bone mineral density and serum adipokines in c57bl/6 male mice. Folia Biologica Praha. 2011;57:133-138.

105. Cho ES. The Effects of rosiglitazone on osteoblastic differentiation, osteoclast formation and bone resorption. Mol and cells. 2012;33:173-181.

106. Kang JH. Involvement of prolyl hydroxylase domain protein in the rosiglitazone-induced suppression of osteoblast differentiation. PLoS One. 2015.

107. Urman A. Inflammation beyond the Joints: rheumatoid arthritis and cardiovascular disease. Sci Fed J Cardiology. 2018;2:1000019.

108. Karmakar S, Kay J, Gravallese EM. Bone damage in rheumatoid arthritis-mechanistic insights and approaches to prevention. Rheum Dis Clin of North Am. 2010;36:385-404.

109. Neumann E, Gay S, Ladner UM. The RANK/RANKL/ osteoprotegerin system in rheumatoid arthritis: New insights from animal models. Arthritis and Rheum. 2005;52:2960-2967.

110. Haynes DR. Osteoprotegerin and receptor activator of nuclear factor kappaB ligand (RANKL) regulate osteoclast formation by cells in the human rheumatoid arthritic joint. Rheum. 2001;40:623-630.

111. Walsh NC. Osteoblast function is compromised at sites of focal bone erosion in inflammatory arthritis. J Bone and Miner Res. 2009;24:1572-1585.

112. Yao Z. Tumor necrosis factor-alpha increases circulating osteoclast precursor numbers by promoting their proliferation and differentiation in the bone marrow through up-regulation of c-Fms expression. J Bio Chem. 2006;281:11846-11855.

113. Jilka RL. Osteoblast programmed cell death (apoptosis): modulation by growth factors and cytokines. J Bone and Min Res. 1998;13:793-802.

114. Sozen T, Ozisik L, Basaran NC. An overview and management of osteoporosis. European J Rheum. 2017;4:46-56.

115. Alswat KA. Gender disparities in osteoporosis. J Clin Medicine Res. 2017;9:382-387.

116. Robinson LJ. Estrogen Inhibits RANKL-stimulated Osteoclastic differentiation of human monocytes through estrogen and RANKL-regulated interaction of estrogen receptor-α with BCAR1 and Traf6. Experimental Cell Res. 2009;315:1287-1301.

117. Jilka RL. Increased osteoclast development after estrogen loss: mediation by interleukin-6. Sci.1992;257:88-91.

118. Fujiwara Y. RANKL (receptor activator of nfκb ligand) produced by osteocytes is required for the increase in b cells and bone loss caused by estrogen deficiency in mice. J Biol Chem. 2016;291:24838-24850.

119. Almeida M. Estrogens and androgens in skeletal physiology and pathophysiology. Physiological Rev. 2017;97:135-187.

120. Obrien CA. Glucocorticoids act directly on osteoblasts and osteocytes to induce their apoptosis and reduce bone formation and strength. Endocr. 2004;145:1835-1841.

121. Liu Y. Effects of dexamethasone, celecoxib, and methotrexate on the histology and metabolism of bone tissue in healthy Sprague Dawley rats. Clin Interv Aging. 2015;10:1245-1253.

122. Liu YZ. Glucocorticoid-induced delayed fracture healing and impaired bone biomechanical properties in mice. Clin Interv in Aging. 2018;13:1465-1474.

123. Briot K, Roux C. Glucocorticoid induced osteoporosis. Rheum and Musc Dis Open. 2015.

124. Ohnaka K. Glucocorticoid suppresses the canonical wnt signal in cultured human osteoblasts. Biochem Biophys Res Commun. 2005;329:177-181.

125. Wang FS. Secreted frizzled-related protein 1 modulates glucocorticoid attenuation of osteogenic activities and bone mass. Endocr. 2005;146:2415-2423.

126. Swanson C. Glucocorticoid regulation of osteoclast differentiation and expression of receptor activator of nuclear factor-kappaB (NF-kappaB) ligand, osteoprotegerin, and receptor activator of NF-kappaB in mouse calvarial bones. Endocr. 2006;147:3613-3622.

127. Reichenberger E, Chen IP. Craniometaphyseal dysplasia, autosomal dominant. GeneReviews. 1993.

128. Wu B. Craniometaphyseal dysplasia with obvious biochemical abnormality and rickets-like features. Clinica Chimica Acta. 2016;456:122-127.

129. Martin K, Nathwani S, Bunyan R. Craniometaphyseal dysplasia: A review and novel oral manifestation. J Oral Biol Craniofacial Res. 2017;7:134-136.

130. Chen IP. Craniometaphyseal dysplasia mutations in ankh negatively affect human induced pluripotent stem cell differentiation into osteoclasts. Stem Cell Rep. 2017;9:1369-1376.

131. Chen IP. A Phe377del mutation in ANK leads to impaired osteoblastogenesis and osteoclastogenesis in a mouse model for craniometaphyseal dysplasia (CMD). Hum Mol Genet. 2011;20:948-961.

132. https://rarediseases.org/rare-diseases/osteopetrosis/

133. Stark Z, Savarirayan R. Osteopetrosis. Orphanet J Rare Dis. 2009;4:5.

134. Jung YK. Enhanced activation of rac1/cdc42 and mitf leads to augmented osteoclastogenesis in autosomal dominant osteopetrosis type II. JBMR Plus. 2019;3:10070.

135. Biver E. Diagnosis, prevention, and treatment of bone fragility in people living with HIV: a position statement from the swiss association against osteoporosis. Osteoporosis Inter. 2019.

136. Ofotokun I, Weitzmann MN. HIV and bone metabolism. Discovery Med. 2011;11:385-393.

137. Hileman CO, Eckard AR, McComsey GA. Bone loss in HI-a contemporary review. Curr opin in endocrionol, diabetes and obesi 2015;22:446-451.

138. Raynaud MB. Bone degradation machinery of osteoclasts: An HIV-1 target that contributes to bone loss. Proceedings of the Ntl Academy of Sci of the United States of America. 2018;115:2556-2565.

139. Ruiz GS. Effects of bilirubin and sera from jaundiced patients on osteoblasts: contribution to the development of osteoporosis in liver diseases. Hepatol. 2011;54:2104-2113.

140. Chen W. Osteoblasts from osteoarthritis patients show enhanced susceptibility to Ross River virus infection associated with delayed type I interferon responses. Virol J. 2014.

141. Chen W. Arthritogenic alphaviral infection perturbs osteoblast function and triggers pathologic bone loss. Proceedings of the Ntl Academy of Sci of the United States of America. 2014;111:6040-6045.

142. Maltby S. Osteoblasts are rapidly ablated by virus induced systemic inflammation following lymphocytic choriomeningitis virus or pneumonia virus of mice infection in mice. J Immunol. 2018; 200:632-642.

143. Josse J, Velard F, Gangloff SC. Staphylococcus aureus vs. osteoblast: relationship and consequences in osteomyelitis. Frontiers in Cellular and Infect Microbiol. 2015;5:85.

144. Pacios S. Bacterial infection increases periodontal bone loss in diabetic rats through enhanced apoptosis. The American J Pathol. 2013;183:1928-1935.

145. Khan A. Bisphosphonate-associated osteonecrosis of the jaw. Canadian Family Physician. 2008;54:1019-1021.

146. Kim KM. Medication related osteonecrosis of the jaw: 2015 position statement of the Korean society for bone and mineral research and the korean association of oral and maxillofacial surgeons. J Bone Metabol. 2015;22:151-165.

147. Radi ZA, Khan NK. Effects of cyclooxygenase inhibition on bone, tendon, and ligament healing. Inflam Res: Official J Euro Hista Res Soci. 2005;54:358-366.

148. Zhang X. Cyclooxygenase-2 regulates mesenchymal cell differentiation into the osteoblast lineage and is critically involved in bone repair. J Clin Investigation. 2002;110:1211.

149. Maxis K. The shunt from the cyclooxygenase to lipoxygenase pathway in human osteoarthritic subchondral osteoblasts is linked with a variable expression of the 5-lipoxygenase-activating protein. Arth Res Ther. 2006;8:R181.

150. Kluesner NH. Miller DG. Scurvy: malnourishment in the land of plenty. J Emergency Med. 2014;46:530-532.

151. Leger D. Scurvy. Canadian Family Physician. 2008;54:1403-1406.

152. Aghajanian P. The roles and mechanisms of actions of Vitamin C in bone: new developments. J Bone and Mineral Res. 2015;30:1945-1955.

153. Niture SK. Nrf2 signaling and cell survival. Toxicol Appl Pharmacol. 2010;244:37-42.

154. PubChem, Ascorbic acid 2019.

155. Leboy PS, et al. Ascorbic acid induces alkaline phosphatase, type X collagen, and calcium deposition in cultured chick chondrocytes. J Biol Chem. 1989;264:17281-17286.