Clinical Reviews in Bone and Mineral Metabolism (2018) 16:95–102
https://doi.org/10.1007/s12018-018-9250-3

Bringing Attention to Lesser-known Bone Remodeling Pathways

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Published online: 1 September 2018

Abstract

Osteoporosis, a disease of low bone mass, places individuals at enhanced risk for fracture, disability, and death. In the USA, hospitalizations for osteoporotic fractures exceed those for heart attack, stroke, and breast cancer and, by 2025, the number of fractures due to osteoporosis is expected to rise to nearly three million in the USA alone. Pharmacological treatments for osteoporosis are aimed at stabilizing or increasing bone mass. However, there are significant drawbacks to current pharmacological options, particularly for long-term management of this chronic condition. Moreover, the drug development pipeline is relatively bereft of new strategies. Consequently, there is an urgent and unmet need for developing new strategies and targets for treating osteoporosis. Casual observation led us to hypothesize that much of the bone remodeling research literature focused on relatively few molecular pathways. This led us to perform bibliometric analyses to determine the relative popularity of bone remodeling pathways in publications and US National Institutes of Health funding of the last 10 years. In this review article, we discuss these findings and highlight several less-examined signaling pathways that may hold promise for future therapies.

Keywords Osteoporosis · Bone remodeling · Cell signaling · Osteoblast · Osteoclast

Introduction

Bone mass in humans generally declines after age 30 due to the rate of bone resorption exceeding the rate of bone formation [1]. Osteoporosis is a disease of low bone mass that places individuals at enhanced risk for fracture, disability, and death [2]. According to the World Health Organization, there are up to 49 million individuals with osteoporosis in North America, Europe, Japan, and Australia alone [3]. Data collected by the US Centers for Disease Control and Prevention between 2005 and 2010 reveal that 16.2% of American individuals over the age of 65 have osteoporosis [4]. In the USA, hospitalizations for osteoporotic fractures exceed those for heart attack, stroke, and breast cancer [5]. It has been estimated that by 2025 the number of fractures due to osteoporosis will increase to nearly three million in the USA alone, creating a $25 billion financial burden [6].

Pharmacological treatments for osteoporosis are aimed at stabilizing or increasing bone mass. Each takes advantage of the fact that the skeletal system is exquisitely capable of resorbing existing bone matrix and forming new bone matrix. The most common treatment for osteoporosis is bisphosphonates, which are anti-resorptive agents that reduce the rate of bone loss by inhibiting osteoclast function [7]. While generally effective in most patients, there are important contraindications to bisphosphonate therapy and, moreover, a drug holiday is recommended after 5 years of treatment due to risk of adverse events such as atypical femoral fracture or osteonecrosis of the jaw [8]. Another anti-resorptive agent, denosumab, reduces osteoclast differentiation by neutralizing RANK ligand, thereby reducing the overall rate of bone resorption [9]. However, much like bisphosphonates, a drug holiday is also recommended after 5 years of denosumab therapy [10].

Despite the noted effectiveness of anti-resorptive therapies, they generally do not increase bone formation but merely slow the rate of bone resorption. For some patients, however, anti-resorptive therapies are unsuccessful in stabilizing bone mass and, moreover, some patients present to clinic with very high fracture risk. An anabolic therapy is advisable in these situations [7] and, in the USA, there are two drugs approved for

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12018-018-9250-3) contains supplementary material, which is available to authorized users.

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osteoporosis treatment: teriparatide and abaloparatide. Teriparatide is a recombinant form of parathyroid hormone (PTH) and is approved for patients for whom other osteoporotic therapies have failed or who are at extraordinarily high risk of fracture [6]. While teriparatide is very effective in increasing bone mass, it can only be administered for 2 years before the treatment must be permanently halted due to a risk of developing osteosarcoma. Moreover, there is a significant rebound effect resulting in bone mineral density (BMD) loss after termination of anabolic therapy, thus requiring patients be placed on an anti-resorptive medication to preserve gains in bone mass [10]. Teriparatide is also an expensive relative to anti-resorptive therapies and therefore difficult for many patients to access. The other bone anabolic drug, abaloparatide, is a modified recombinant PTH-related peptide (PTHrP) that is quite effective at increasing bone mass but, like teriparatide, is only approved for a treatment period of 2 years and cannot be administered to patients who have received teriparatide as a treatment (and vice versa).

Thus, despite the fact that osteoporosis rates are expected to rise significantly in the coming decades [11], there are limited pharmacological treatment options, particularly for long-term management of this chronic condition. Moreover, the drug development pipeline is relatively bereft of new strategies; for instance, to the best of our knowledge, the only candidate anabolic drug currently in clinical trial for osteoporosis is a biosimilar to teriparatide (PF708, Pfenex Inc.). Additionally, several promising candidate therapies with novel mechanisms of action while effective at improving bone mass and reducing fracture incidence have been associated with significant adverse events in clinical trials [12, 13]. Some adverse events were found significant enough to pull seemingly promising drugs out of development, as seen in the example of odanacatib, which is a cathepsin K inhibitor halted due to increased risk of stroke in premenopausal women (Merck & Co. Website, retrieved on August 10, 2018). Consequently, there is an urgent and unmet need for developing new strategies and targets for treating osteoporosis. That said, casual observation led us to hypothesize that much of the bone remodeling research literature focused on relatively few molecular pathways. This led us to perform bibliometric analyses to determine the relative popularity of bone remodeling pathways in publications and US National Institutes of Health (NIH) funding over the last 10 years. In this review article, we discuss these findings and highlight several less-examined signaling pathways that may hold promise for future therapies.

Identifying Popular Pathways in Bone Remodeling

A literature search was performed in PubMed.gov using the search term ("skeleton" or "bone") and ("signaling" or "pathway") with results restricted to the 10-year period between January 1, 2008, and December 31, 2017. This yielded a total of 29,378 publications. We then took the text of the abstracts from the 10,000 most recent publications and determined frequently found terms using an online word frequency counter (http://www.writewords.org.uk/word_count.asp). Using the term "bone" as our reference (26,365 appearances), we identified terms relating to signaling pathways appearing at least 50 times (i.e., 0.002 frequency relative to "bone"), which yielded a total of 151 terms (Supplemental Table 1); the most and least frequent pathway-related terms were "bmp" (0.25 relative to bone) and "tbx" (0.0022 relative to bone), respectively.

We then combined terms relating to the same signaling pathway to identify popular pathways in the field (Supplemental Table 2). Adding these terms to the search revealed that approximately 50% of publications from the last 10 years mention or discuss just three pathways—transforming growth factor-beta (TGF-β) superfamily (31.34%), mitogen-activated protein (MAP) kinase (13.72%), and Wnt (13.21%). To more narrowly examine the skeletal literature, we included the search term "'osteoblast' or 'osteocyte' or 'osteoclast,'" which revealed these three pathways in > 55% of publications from this time period (2699 out of 4826 publications); the relative popularity of pathways in the field is detailed in Supplemental Table 3 and of the top 50 pathways in Fig. 1. We were interested if the popularity of these pathways among publications relates to popularity among grants funded by the US NIH in the bone remodeling field (Fig. 1 and Supplemental Table 4). Consistently, more than 46% of funded grants in the bone remodeling field from 2008 to 2017 (862 out of 1850) mention or discuss the TGF-β superfamily, MAP kinase, or Wnt signaling pathways in their abstract; including keywords relating to parathyroid hormone (PTH) in this search retrieves nearly 55% of funded grants (1012 out of 1850). To us, this indicates a rather striking lack of heterogeneity among pathways studied in the bone remodeling field.

Lesser-Studied Pathways in Bone Remodeling

In order to identify lesser-studied pathways in bone remodeling, we excluded pathways with 50 or greater publications in the last 10 years. Then, to identify particularly notable pathways for the focus of this review article, we generated the following inclusion criteria: (1) functional evidence (knockout, pharmacological, etc.) published in a peer-reviewed journal and indexed in PubMed.gov, (2) fewer than ten review articles published about the pathway in the skeleton in the last 10 years, and (3) identifiable as a distinct signaling pathway (rather than a downstream effector such as phospholipase C). This refinement resulted in a short list of lesser-studied, yet distinct, signaling pathways implicated in bone remodeling;

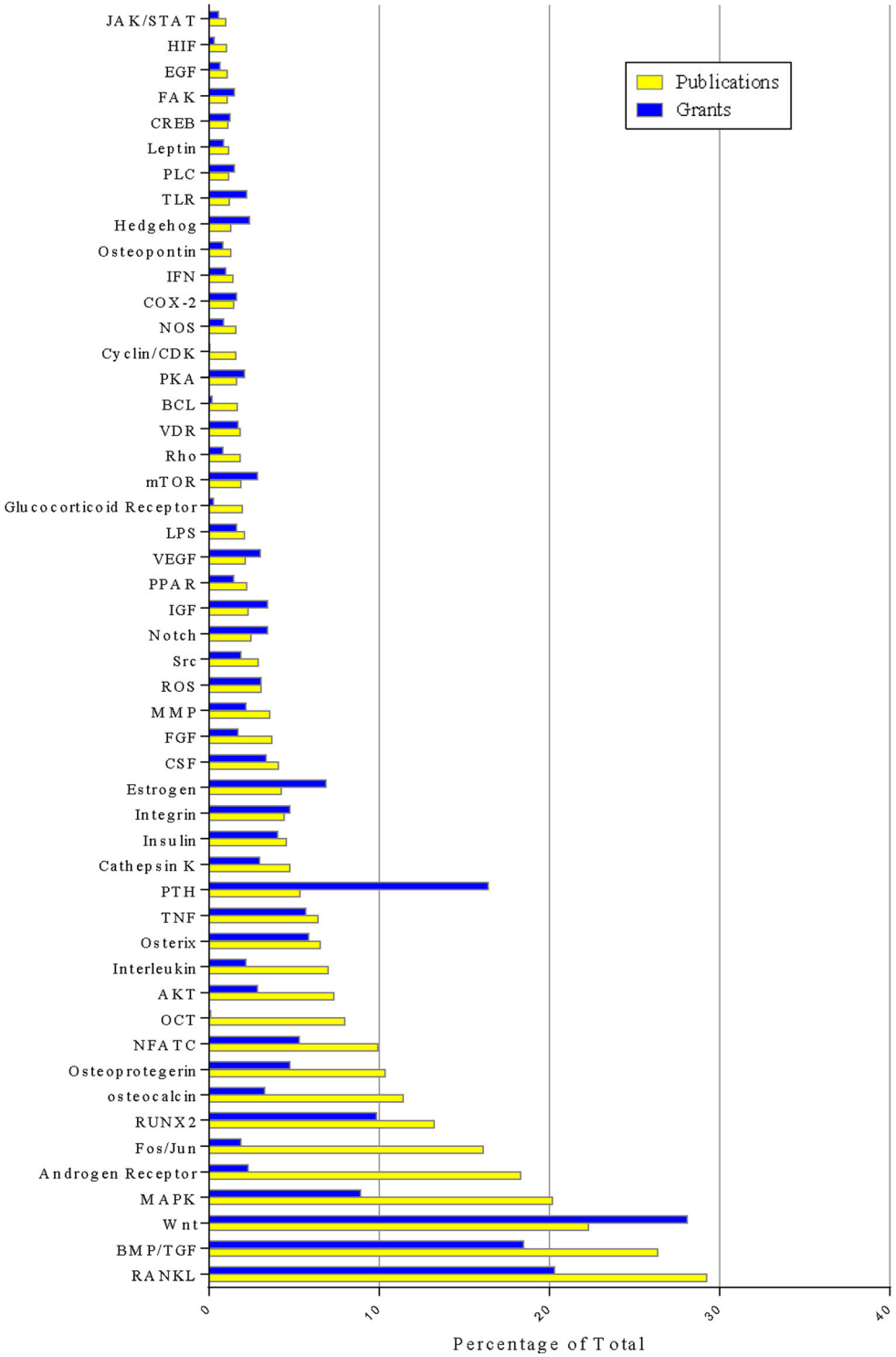

Fig. 1 Fifty most popular pathways in publications and funded grants (indexed in PubMed.gov and NIH Reporter, respectively) from January 1, 2008, to December 31, 2017, as identified using the search terms detailed in Supplemental Table 2 in combination with the search "skeleton" or "bone" and "signaling" or "pathway" and "osteoblast" or "osteocyte" or "osteoclast"

the reported evidence for these pathways is discussed in the following sections.

Apolipoprotein D

Global *Apolipoprotein D* (*ApoD*) knockout mice exhibit low bone mass in both trabecular and cortical compartments of the femur that is associated with increased bone turnover rate [14]. The phenotype appears to be stronger in females than males and is also more severe in older mice [14]. While the precise molecular mechanism underlying this phenotype is not clear, *ApoD* knockout mice display an increased ratio of *Rankl*:*Opg* and increased osteoclast number [14]. This suggests an alteration in osteoblast-to-osteoclast coupling, which

is consistent with the finding that *ApoD* expression correlates with osteoblastic differentiation status in primary human bone marrow stromal cells [15], primary murine calvarial osteoblasts [16], and the murine cell lines C3H10T1/2 and MC3T3-E1 [14, 17]. Moreover, osteoblastic differentiation is impaired in bone marrow stromal cell obtained from *ApoD* knockout mice and this defect is reduced by exogenous ApoD [14]. The immediate translational potential for this pathway is unclear, however, as overexpression of *ApoD* from the neuronal human *Thy-1* promoter does not influence bone mass in either male or female mice [14].

Aryl Hydrocarbon Receptor

The ligand-activated transcription factor aryl hydrocarbon receptor (AhR) aids in regulating the downstream biological responses to aromatic hydrocarbons, such as those commonly found in cigarette smoke. During inactivity, AhR is bound to numerous chaperone proteins in the cytoplasm. In the presence of aromatic hydrocarbons, ligand-bound AhR dissociates from chaperone proteins, translocates to the nucleus, and dimerizes with AhR nuclear translocator to affect gene transcription. AhR is expressed in both osteoblasts and osteoclasts [18] while global *AhR* knockout mice exhibit high bone mass due to reduced resorption [19]. This is consistent with the fact that mice lacking *AhR* in osteoblasts display normal bone mass while mice devoid of osteoclastic *AhR* demonstrate decreased resorption [19]. When challenged by an AhR agonist, 3-methylcholanthrene, mice lacking osteoclastic *AhR* were protected from carcinogen-mediated bone loss [20]. In vitro treatment with 3-methylcholanthrene in multiple bone cell lines increases expression of estrogen metabolizing and synthesizing enzymes, such as *Cyp1b1* and *aromatase*. Subsequent antibody cytokine analysis found that expression levels of interleukin-1β and interleukin-6 were increased by 3-methylcholanthrene and these interleukins are well known to induce *aromatase* [18].

Exposure to carcinogens such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or "dioxin") inhibits spinal fusion in rats and even after prolonged termination of dioxin, only partial bone-healing capacity is restored [21]. In primary rat bone marrow stromal cells, dioxin exposure inhibits alkaline phosphatase activity and matrix mineralization; however, co-treatment with AhR antagonists lessened these effects [22]. Dioxin-mediated activation of AhR dose dependently suppressed the expression of osteoblastic markers [23].

Similarly, the prototypical aromatic hydrocarbon, benzo(a)pyrene, limits tibial fracture repair [24] and this effect can be abrogated by the natural AhR antagonist resveratrol in both in vitro and in vivo models [25]. Accelerated osteoclast differentiation can be driven by ligand activation of AhR by benzo(a)pyrene in a receptor-dependent manner [26]. Mechanistically, benzo(a)pyrene activates AhR signaling while simultaneously inhibiting the TGF-β1/SMAD4 and TGF-β1/ERK/AKT signaling pathways [25].

In addition to carcinogens, arthritis is known to induce AhR signaling. In a mouse model of collagen-induced arthritis, AhR expression is increased and correlates with decreased bone mineral density. High expression levels of *AhR* were observed in osteoblasts and correlated with the suppression of osteoblastic markers including *Runx2* and *Alp* [23]. Comparably, immunofluorescence staining showed that high expression of AhR was localized in osteoblasts from the collagen-induced arthritis mice [27]. In vitro studies in the pre-osteoblast cell line MC3T3-E1 demonstrated activation of AhR inhibited cellular proliferation and differentiation in a dose-dependent manner.

Lysophosphatidic Acid

Lysophosphatidic acid (LPA) is a bioactive phospholipid that promotes osteoclast differentiation and/or fusion of osteoclast precursors and increases osteoclast cell survival in vitro [28–30]. LPA also promotes osteoblastic differentiation of primary bone marrow stromal cells and MC3T3-E1 cells [31–34]. That said, these outcomes are likely due to integration of numerous LPA-mediated actions since LPA signals via several heterotrimeric G protein-coupled receptors whose effects may be antagonistic. For instance, in human bone marrow stromal cells, LPA signaling via LPA receptor 1 (LPA1) promotes osteoblastogenesis while LPA signaling via LPA receptor 4 (LPA4) restricts osteoblastogenesis [34, 35]. Consistent with this observation, global *LPA1* knockout mice display low trabecular bone mass in the femur while global *LPA4* knockout mice have high trabecular bone mass at this site [34, 35]. To the best of our knowledge, the cellular and molecular mechanism(s) mediating these disparate phenotypes has not been reported.

Osteoclast Inhibitory Peptide-1

Osteoclast inhibitory peptide-1 (OIP-1) is a glycophosphatidylinositol-linked membrane protein that contains a carboxy-terminal GPI-linked peptide "c-peptide," which is important for the inhibition of osteoclasts [36]. OIP-1 binds the Fc gamma receptor IIB to inhibit osteoclast differentiation [37]. An osteopetrotic bone phenotype occurs in a transgenic model of OIP-1/hSca expression in osteoclast-linage cells [38]. Specifically, *OIP-1* transgenic (Tg) mice demonstrate increased bone mineral density, bone mineral content, trabecular thickness, and bone volume in the humerus and lumbar spine. Bone marrow cultures from *OIP-1* Tg mice exhibit decreased osteoclast progenitors along with suppression of TRAF-2, c-Fos, p-c-Jun, and NFATc1 protein levels after RANKL stimulation [38]. Pre-osteoclasts from *OIP-1* Tg mice express higher activation levels of immunoreceptor

tyrosine-based inhibitory motif phosphorylation of Fc gamma RIIB, the receptor OIP-1 binds on osteoclasts. Spleen tyrosine kinase activation is also inhibited in *OIP-1* Tg mice compared to wild-type controls, raising the possibility that a cross regulation of immunoreceptor tyrosine-based inhibitory motif and Fc gamma RIIB receptors could contribute to OIP-1's suppression of osteoclast differentiation and spleen tyrosine kinase activation [37]. Finally, there may be therapeutic potential in this pathway as treatment of peripheral blood from Paget's patients with OIP-1 c-peptide decreases osteoclast differentiation [36].

Oxytocin

Oxytocin is a nonapeptide hormone synthesized by the posterior pituitary gland that exerts both central and peripheral signaling effects through binding to the oxytocin receptor (OTR), which is a heterotrimeric G protein-coupled receptor. Both global *oxytocin* and *OTR* knockout mice display low femoral bone mass due to impaired bone formation rate and reduced osteoblast number [39]. Several lines of evidence indicate this effect is due to direct action of oxytocin on osteoblasts and/or osteoblast precursors: first, oxytocin promotes osteoblastic differentiation of osteogenic cells in vitro [39, 40] and this effect requires OTR expression [41]; second, delivery of oxytocin via ventricular injection does not impact bone mass whereas its systemic delivery increases bone mineral density [39]; and finally, OTR is expressed in osteoblasts [39] and its specific deletion in these cells leads to low bone mass [42]. Moreover, this pathway may hold translational potential for treating postmenopausal bone loss since systemic administration of oxytocin (or an analog) reverses bone loss in ovariectomized mice [40].

It should be mentioned that oxytocin also promotes osteoclastic differentiation in vitro [39] and osteoclastic differentiation potential is diminished in pregnant mice with global *oxytocin* deletion [43]. However, these findings must be balanced with data indicating that, in nonpregnant conditions, osteoclast number is unchanged in global *oxytocin* knockout mice [39] and, moreover, bone mass is normal in mice with osteoclast-specific deletion of *OTR* [42].

Taste Receptor Type 1 Family

The taste receptor type 1 (Tas1R) family of heterotrimeric G protein-coupled receptors consists of three members: Tas1R1, Tas1R2, and Tas1R3 [44]. Tas1R3 is a bifunctional receptor in that it recognizes amino acids when dimerized with Tas1R1 or sweet molecules such as glucose when dimerized with Tas1R2, either of which leads to activation of a common signaling response involving alpha-gustducin-mediated activation of phospholipase C-beta2 [44]. Thus, Tas1R family members are generally regarded as nutrient sensors that monitor energy and nutrient status in the extracellular environment. Though most closely associated with gustatory tissues, Tas1R family members are also found in numerous extraoral tissues including the gastrointestinal tract, brain, bladder, pancreas, male reproductive organs, immune system, adipose tissue, and bone [44, 45]. We are unaware of data regarding Tas1R1 function in the skeleton; however, global knockout of either *Tas1R2* or *Tas1R3* leads to modest increase in bone mass in trabecular and cortical compartments of long bones [46]; a second study corroborates the high cortical bone mass in tibiae of global *Tas1R3* knockout mice [45]. High bone mass in *Tas1R3* knockout mice is associated with decreased serum levels of the bone resorption marker collagen type I C-telopeptide [45]. Consistent with a role in osteoclast function, *Tas1R3* and its putative partner *Tas1R2* are expressed in primary osteoclasts and their expression levels positively correlate with differentiation status [45]. No changes in osteoblast-related parameters were reported in *Tas1R3* or *Tas1R2* knockout mice. Collectively, these findings suggest that nutrient sensing by the Tas1R3:Tas1R2 heterodimer in osteoclasts regulates bone resorption. This idea is consistent with the fact that restricting the concentration of glucose, which is a candidate ligand for the Tas1R3:Tas1R2 complex [47], leads to impaired osteoclast activity in vitro [48]. That said, the global nature of these knockout mouse lines makes it impossible to rule out the possibility that defects in other physiological contexts underlie the high bone mass phenotype; future studies involving conditional knockout mice in a cell type-specific manner are required to better characterize the role of Tas1R family members in postnatal bone remodeling.

Miscellaneous

In addition to those discussed above, we identified a few pathways for which there are substantially fewer reported data (albeit with in vivo functional evidence) implicating a role in bone remodeling. We briefly highlight those pathways here and suggest that follow-up studies—especially replication studies and/or conditional knockout models—would be helpful in establishing their role in skeletal biology. For instance, neuromedin-U (NMU) is a neuropeptide responsible for a variety of central and peripheral activities including regulation of blood pressure and smooth muscle contraction [49]. Additionally, a single publication from 2007 reports that global homozygous knockout of *NMU* leads to high trabecular bone mass in femora due to increased bone formation rate [50]; however, it is unclear if this is due to a central or peripheral action of NMU since its ability to alter osteoblast behavior is controversial [50, 51].

Another example is the receptor tyrosine kinase Tyro3, global homozygous deletion of which leads to high bone mass in tibiae of 10-week-old mice and is associated with reduced osteoclast differentiation in vivo and in vitro [52]. These data are consistent with a prior study that demonstrated Tyro3 activation promotes osteoclast activity in vitro [53]. That said, the global nature of the knockout in *Tyro3* mutant mice leaves open the possibility that reduced bone resorption in these mice is secondary to a defect in another physiological context. This line of investigation could benefit from conditional deletion of *Tyro3*—or the gene encoding its ligand growth arrest-specific protein 6 (Gas6) [54]—in specific skeletal cell types. At the same time, an immediate translational opportunity may exist in that soluble Tyro3, which is commercially available conjugated to the constant fragment of human IgG_1, blocks Gas6-induced osteoclastic differentiation in vitro [52].

The type 1 equilibrative nucleoside transporter (ENT1) is an integral membrane protein that carries out cellular uptake of adenosine [13], thereby impacting the concentration in both the intracellular and extracellular environments. Several studies indicate adenosine signaling via cell surface receptors regulates differentiation of both osteoblasts and osteoclasts [55–61]. There are relatively little data regarding the role of ENT1 in the skeletal cells; however, global knockout of *ENT1* leads to low bone mass in the vertebrae of 7-month-old mice and is associated with increased osteoclast number [62]. The precise molecular mechanism underlying this phenotype is unclear and is likely nuanced as modulation of adenosine signaling by deletion of specific adenosine receptors may lead to either high or low bone mass [60, 61].

Concluding Remarks

There is an urgent and unmet need for developing new strategies and targets for treating osteoporosis. That said, our bibliometric analysis indicates a striking lack of heterogeneity within the bone remodeling field—with just three pathways accounting for more than 50% of publications and nearly 50% of funded NIH grants in this field during the last 10 years. We are concerned that this current lack of diversity may restrict discovery of novel therapeutic approaches and, therefore, encourage investigators to expand into lesser-studied pathways in order to broaden the collective focus of the field. Here, we present brief overviews of several pathways for which functional evidence (genetic, pharmacological, etc.) indicates a role in the regulation of osteoblasts and/or osteoclasts. Additional work is required to elucidate the mechanism(s) by which these pathways intersect with and/or modulate the complicated signal transduction network underlying bone remodeling.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Ethical Approval This article does not contain any studies with human participants or animal subjects performed by the any of the authors.

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