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REVIEW ARTICLE

Regulation of bone and cartilage by adenosine signaling

Lauren C. Strazzulla[1] · **Bruce N. Cronstein**[2]

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Abstract There is growing recognition that bone serves important endocrine and immunologic functions that are compromised in several disease states. While many factors are known to affect bone metabolism, recent attention has focused on investigating the role of purinergic signaling in bone formation and regulation. Adenosine is a purine nucleoside produced intracellularly and extracellularly in response to stimuli such as hypoxia and inflammation, which then interacts with P1 receptors. Numerous studies have suggested that these receptors play a pivotal role in osteoblast, osteoclast, and chondrocyte differentiation and function. This review discusses the various ways by which adenosine signaling contributes to bone and cartilage homeostasis, while incorporating potential therapeutic applications of these signaling pathways.

Keywords Adenosine receptors · Bone metabolism · Purinergic signaling · Chondrocytes

The components of bone

Although bone has traditionally been viewed as simply providing mechanical support, it is now appreciated that bone serves a number of more complex functions. In addition to providing the venue for production of white and red blood cells, and serving as a reservoir for calcium and phosphorous, bone is now recognized as an endocrine organ producing growth factors such as fibroblast growth factor 23 (FGF23) [1]. Formation of bone may occur through two processes: intramembranous osteogenesis where bone is formed directly and endochondral osteogenesis which requires a cartilage template that is subsequently replaced by bone [2].

Bone is also a dynamic organ that is remodeled in response to both local and systemic processes such as bone microdamage, changes in mechanical load, hormonal disruptions, immune mediators, and growth factors. This tissue is comprised of three principal effector cell types: osteoblasts, osteocytes, and osteoclasts [2]. Osteoblasts are derived from mesenchymal progenitor cells, which also have the potential to differentiate into adipocytes, chondrocytes, fibroblasts, and myocytes [3]. Osteoblasts generate bone tissue by producing matrix proteins such as type I collagen, osteonectin, and proteoglycans. These components form osteoid, which eventually undergoes mineralization [4–7]. Importantly, osteoblasts control osteoclast differentiation through expression of both surface and soluble receptor activator of NFkB Ligand (RANKL), a critical osteoclast differentiation factor, and through production of osteoprotegerin (OPG), a soluble receptor of RANKL which prevents the interaction of RANKL with its receptor [8–10]. Indeed, the RANKL/OPG ratio is a major determinant of bone mass [11]. As osteoblasts form new osteoid, they become embedded in the new matrix and further differentiate into osteocytes. Osteocytes, which are derived from osteoblasts, act as mechanotransducers and react to changes in the mechanical load on the bone [12].

Osteoclasts, on the other hand, are multinucleated cells that form when myeloid progenitors fuse. The elaboration of two distinct molecular signals, monocyte colony stimulating factor (M-CSF) and RANKL, and their binding to receptors on osteoclast precursors is required for the differentiation of osteoclasts [13]. These proteins are elaborated in the

✉ Bruce N. Cronstein
Bruce.Cronstein@nyumc.org

[1] Department of Medicine, School of Medicine, New York University, New York, NY 10016, USA

[2] Divisions of Rheumatology and Translational Medicine, Department of Medicine, School of Medicine, New York University, 550 First Avenue, MSB251, New York, NY 10016, USA

microenvironment by osteoblasts and stromal cells, and removal of these cells leads osteoclasts to rapidly undergo apoptosis [14]. Osteoclasts have the unique ability to resorb bone extracellularly by excavation of pits and troughs on bone surfaces [15]. This process occurs primarily through the direct action of osteoclasts which migrate into the site of resorption, attach to bone, and create a sealed extracellular vacuole into which protons dissolve bone mineral and enzymes degrade the collagenous organic matrix [15]. Bone resorption is an important process for calcium and mineral homeostasis, fracture repair, and to adapt to changes in the mechanical load on bone tissue [1].

Adenosine signaling

It has been recognized since 1929 that adenosine is a biologically significant molecule that regulates multiple systems including, among many others, cardiac conduction, arterial pressure, and intestinal motility [16]. Adenosine exerts its physiologic effects by activating cell surface G protein-coupled receptors including A1, A2A, A2B, and A3 [17]. Considerable progress has been made in recent years to show that adenosine plays an integral role in bone development. The first report of adenosine as a mitogen for bone came in 1995, when it was noted to increase DNA synthesis and cell proliferation in MC3T3-E1 (osteoblast precursor) cells [18]. Further evidence for the potential role of adenosine in regulation of bone came from the observation that patients with adenosine deaminase (ADA) deficiency, in which adenosine levels are elevated more than 10-fold, have multiple skeletal abnormalities including unusual scapular spurring and anterior rib cupping. These abnormalities resolve following 6–12 months of ADA enzyme replacement which presumably normalizes adenosine levels [19]. Deletion of ADA in mice leads to low trabecular bone volume, reduced numbers of trabeculae, as well as decreased expression of RANKL [20].

Adenosine is a nucleoside that is produced both intracellularly and extracellularly through enzymatic degradation of adenine nucleotides [21, 22] (see Fig. 1). The very short half-life of adenosine in blood and other fluids, typically measured in seconds, limits the activity of adenosine as an extracellular signal and maintenance of adenosine levels in extracellular fluids is the result of an equilibrium between production and consumption [23]. A basal level of extracellular adenosine is maintained between 30 and 200 nM [24, 25]. Extracellular adenosine is cytoprotective, increases oxygen supply, angiogenesis, and protects against ischemic damage [26]. Extracellular adenosine synthesis is controlled by ectonucleotidases located on the plasma membrane, including ectonucleoside triphosphate diphosphohydrolase 1 (CD39), ecto-5′nucleotidase (CD73), nucleotide pyrophosphatase phosphodiesterase 1 (NPP-1) and tissue non-specific alkaline phosphatase (TNAP). These enzymes raise adenosine concentrations by hydrolyzing extracellular ATP to ADP, AMP, and adenosine. Without these enzymes, bone development fails to progress normally. For example, CD73 knockout mice are osteopenic and have diminished osteoblast function [27].

Extracellular adenosine concentrations vary according to physiologic and pathologic stimuli such as hypoxia and inflammation [28]. An important source of adenosine is the breakdown of adenine nucleotides which can be released from cells by several different processes. First, apoptotic or necrotic cells release high levels of ATP, the most abundant molecule in the cell [29]. Nucleotide release can also occur in a controlled manner through membrane ion channels such as connexin hemichannels, pannexins, and stretch and voltage-activated channels [30, 31]. Facilitated diffusion may also occur via a nucleotide-specific ATP-binding cassette transporter. Finally, vesicular exocytosis is an important mediator of ATP release from osteoblasts [32]. Release of ATP in bone has been shown to depend on the differentiation state of the cell with mature osteoblasts releasing several fold more ATP than undifferentiated cells [32, 33]. Hormones and neurotransmitters are also thought to regulate ATP release into the extracellular space [34, 35].

Adenosine uptake by cells and tissues from the local environment also plays an important role in regulating purinergic signaling. There are four known nucleoside transport proteins in human cells (hENT1–4) that facilitate cellular uptake of nucleosides from the surroundings [36]. In both humans and mice, equilibrative nucleoside transporter 1 (ENT1) is widely expressed and responsible for the majority of adenosine transport across the plasma membrane [36]. Knockout mice lacking ENT1 display reduced adenosine uptake and increased circulating levels of adenosine in the plasma [37, 38]. Interestingly, these mice show evidence of ectopic bone mineralization with involvement of the spine and sternal fibrocartilaginous tissue, a condition which is similar to lesions seen in diffuse idiopathic skeletal hyperostosis (DISH) [39, 40]. Similarly, individuals lacking ENT1 (homozygous for a null mutation in *SLC29A1*) suffer from ectopic mineralization suggesting the role of ENT1 in bone metabolism in vivo [41]. In 2014, Hinton et al. investigated the bone characteristics in ENT1 null mice and observed abnormal changes including decreased bone density in the lower half of the spinal cord and femur, increased markers of osteoclast activity in the femur, and increased bone density in the cervical and upper thoracic vertebrae [42]. These researchers speculated that the dysregulation of bone density may be secondary to disruption of adenosine signaling. Nonetheless, the changes in bone density observed in ENT1 null mice may be related to age, as aberrant bone density was only found in older ENT1 null mice [42] and this phenomenon may be related to altered expression of adenosine receptors as other studies have shown an age related decline of A1R but not A2R [43]. Another potential

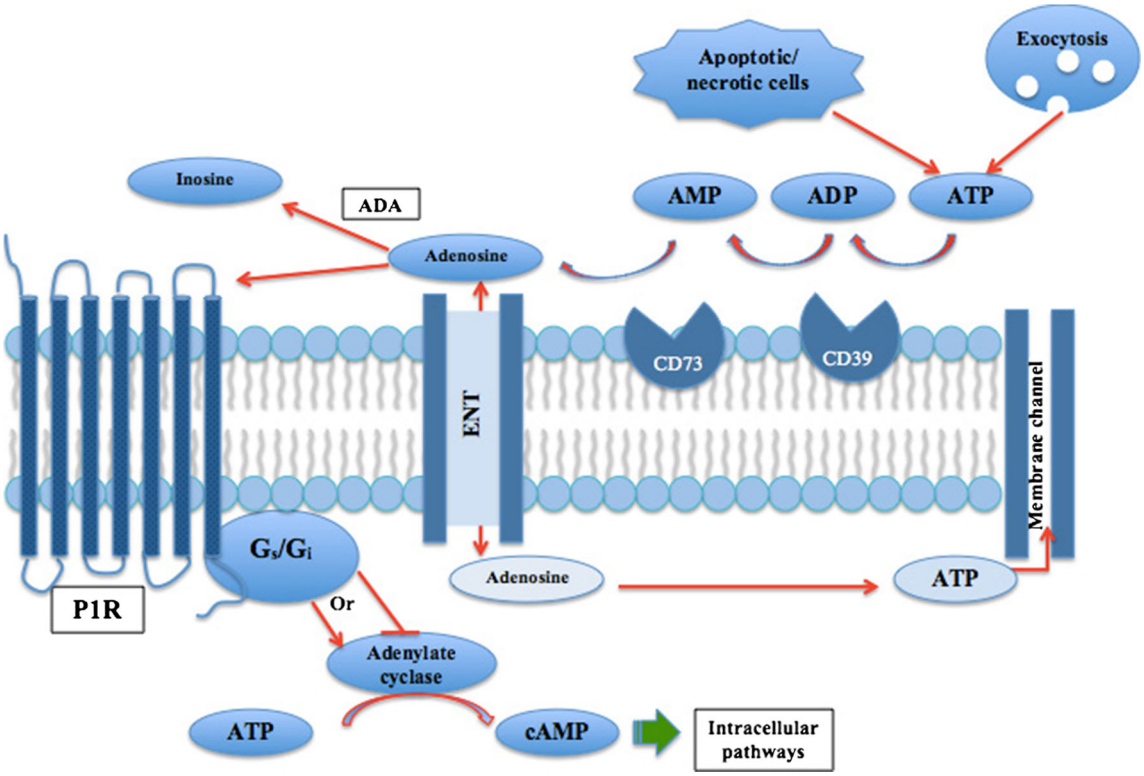

Fig. 1 Adenosine metabolism in the cell. ATP is released into the extracellular space from damaged cells, vesicular exocytosis, and through membrane ion channels including connexins and pannexins. Adenosine is then produced from dephosphorylated ATP via cytosolic ecto 5′nucleotidases CD39 and CD73, and transported through the lipid bilayer via equilibrative nucleoside transporters (ENTs). Adenosine signals through a purinergic receptor (A1, A2A, A2B, A3), which is coupled negatively or positively to adenylate cyclase and the resulting activation or inhibition of cAMP then affects downstream intracellular pathways

explanation for this phenomenon is that high adenosine levels desensitize adenosine receptors involved in regulation of osteoclast and osteoblast function.

Adenosine receptors

In 1976, two subfamilies of purinergic receptors were identified: P1 and P2 receptors [44]. At present, there are four P1 adenosine receptors in vertebrates—A1, A2A, A2B, A3. These receptors are broadly divided into two subclasses: those that are negatively coupled to (A1 and A3) or stimulate (A2A and A2B) adenylate cyclase [17], and within the A2 subclass there are both high affinity (A2AR) and low affinity (A2BR) subtypes [45]. In 2006, Evans et al. showed for the first time that adenosine is formed by osteoprogenitor cells at a high enough concentration to stimulate adenosine receptors. Using a human osteoprogenitor cell line (HCC1) and primary bone marrow stromal cells they demonstrated expression of messenger RNA (mRNA) for CD73, adenosine deaminase, adenosine kinase, and A1, A2A, A2B, and A3 receptors. Prior to this study, little was known about the expression of adenosine receptors by bone cells [21]. By regulating intracellular cAMP levels, these receptors impact other signaling pathways including mitogen-activated protein kinases (MAPKs), and serine-threonine-specific kinases [46]. Adenosine receptors are involved in signaling processes that affect multiple systems. In fact, mutations in these receptors have been implicated in a number of conditions ranging from aspirin intolerant asthma [47] to infarct size in ischemic cardiomyopathy [48], GI toxicity [49], and even panic disorder [50].

Regulation of osteoclast differentiation and function by adenosine

P1 receptors: A1R

Expression of all four adenosine receptor subtypes has been confirmed in murine bone marrow cells, splenocytes and the murine monocytic cell line RAW264.7. In particular, A1R activation is known to promote human multinucleated giant cell formation and, based on this observation, was postulated to play a role in osteoclast development [51] (see Fig. 2). In support of this hypothesis, osteoclast precursors from A1KO mice form fewer tartrate-resistant acid phosphatase (TRAP) positive multinucleated cells and produce osteoclasts with a reduced ability to form an actin-dependent sealing zone on the

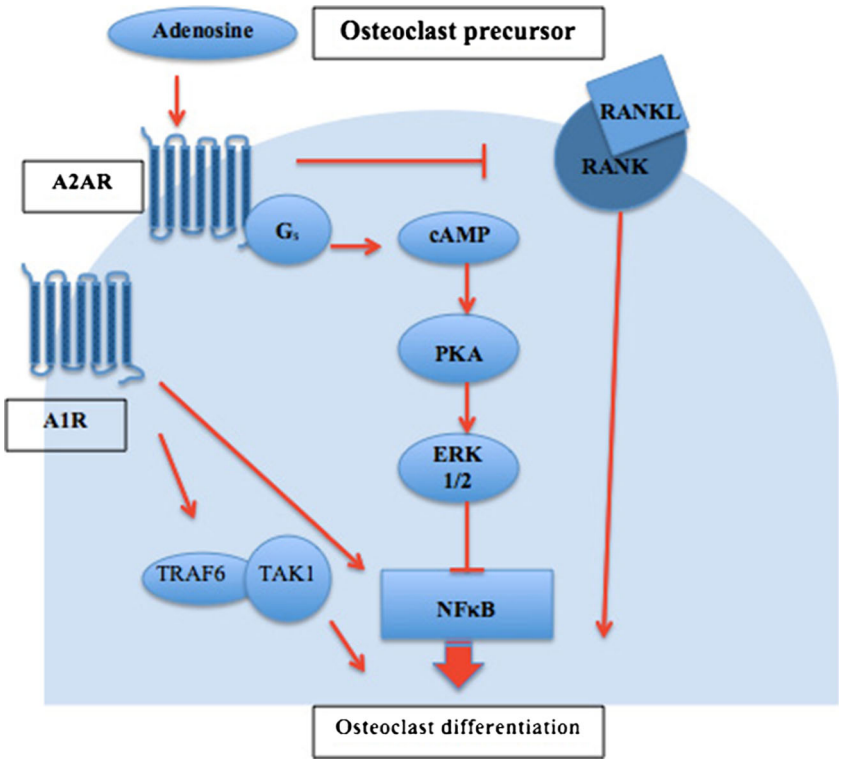

Fig. 2 Role of adenosine receptors in osteoclast differentiation. Activation of A2AR inhibits osteoclast differentiation by blocking NFkB nuclear translocation through activation of the PKA/ERK1/2 pathway. A1R activation promotes osteoclast differentiation by induction of NFkB nuclear translocation, and formation of TRAF6-TAK1 complex. Less is known about the role of A2BR and A3R in osteoclast differentiation

face of bone which is required for bone resorption [51]. By blocking A1R with a selective antagonist, rolofylline, M-CSF/RANKL-induced osteoclast differentiation of bone marrow cells is inhibited in a dose-dependent manner [52]. This likely occurs because A1R activation is needed for signaling at RANK, which, in turn, is needed for activation of NFkB, a requirement for osteoclastogenesis [53]. Downstream of RANK, activation of a complex formed between the signaling molecules TNF receptor associated factor 6 (TRAF6) and TAK1 kinase is required for osteoclastogenesis and this, too, is disrupted when A1R is blocked [53]. In vivo, blockade or deletion of A1R suppresses RANKL-induced NFkB activation, leading to increased bone density and prevention of ovariectomy-induced bone loss [51, 53, 54].

P1 receptors: A2AR

Activation of A2AR opposes the action of A1R on cAMP generation, among other phenomena, and, in contrast, inhibits osteoclast differentiation. Consequently, A2AR deficient mice display an increased number of active osteoclasts in their bones and diminished bone density [55]. Consistent with this in vivo observation, activation of A2AR inhibits M-CSF/RANKL stimulated osteoclast differentiation, and decreases IL-1β and TNF-alpha levels, which may also play a role in inhibiting osteoclast formation. In vitro studies show A2AR stimulation reduces the number of TRAP positive cells and expression of osteoclast differentiation markers [55]. The effect of A2AR on osteoclast differentiation involves signaling through the cAMP/PKA/ERK1/2 pathway [56]. Consistent with the role of cAMP in A2AR signal transduction, Mediero et al. [57] reported that knockdown of the PKA alpha catalytic alpha subunit blocked the inhibitory effect of A2AR on osteoclast differentiation in RAW246.7 cells. Also, A2AR stimulation diminishes ERK1/2 activation which is needed for osteoclast survival and differentiation [58, 59]. However, some studies are not in agreement with these findings. For instance, Pellegatti et al. found that stimulation of the A2A receptor increased fusion of osteoclasts, a key step in their differentiation. Their research also revealed CGS21680, an A2A receptor agonist, could potentiate M-CSF/RANKL stimulated fusion [60]. Importantly, Pellegatti's group used stimulated human peripheral blood monocytes whereas the other studies were carried out in bone marrow derived murine and human osteoclast precursors, which most likely accounts for these differences.

In terms of the therapeutic role of A2AR receptors, in patients with inflammatory bone disease, A2AR agonists reduce bone pitting and loss by decreasing the number of osteoclasts and the degree of inflammation [61]. A more recent study demonstrated that weekly low-dose methotrexate (MTX) injections decreased bone pitting but treatment with ZM241385, an A2AR antagonist, or knockout of A2AR abrogated this effect. MTX was also found to increase bone volume, total volume of bone, and bone mineral density in a model of inflammatory bone disease, but these effects were reversed with

ZM241385 treatment. Similarly, MTX-treated mouse calvaria showed a reduction in the area of inflammation, and in the number of TRAP-positive osteoclasts along with an increase in new bone formation, effects which were also reversed by ZM241385 treatment. A2A receptor stimulation leads to increased osteoprotegerin expression by osteoblasts, reducing RANKL expression and causing inhibition of osteoclast differentiation. Taken together, these findings suggest that MTX treatment reduces inflammatory bone destruction, in this case wear particle-induced osteolysis, through the A2A receptor [62].

P1 receptors: A2BR and A3R

The role of A2B and A3 receptors in osteoclast differentiation is less well defined. For example, while some have found the application of an A2BR-specific agonist, BAY60-6583 in murine bone marrow macrophages inhibits RANKL-induced osteoclast formation in a dose-dependent manner [53], this contrasts with the findings of Termachi et al. who reported that adenosine blocked the MTX-induced osteoclast inhibition via A2BR activation [63]. These effects may be secondary to involvement of the A2BR receptor on osteoblasts, which play a crucial role in stimulating osteoclast differentiation via RANKL.

Activation of the A3 receptor by the highly selective A3R receptor agonist IB-MECA decreases the number of osteoclasts in rats and down regulates PI3K, NFkB, and RANKL [64]. Also, Varani et al. studied the effect of IB-MECA on MRMT-1 rat mammary gland carcinoma cells which are known to cause cancer-associated osteolytic lesions. This agonist reduced NFkB activation, and increased p53 levels. Also, caspase-3 levels and lactate dehydrogenase were increased which suggests a cytotoxic effect of this agonist. Compared to cisplatin, IB-MECA had several advantages, such as causing less weight loss, and not compromising bone marrow functionality. IB-MECA was postulated to reduce bone cancer progression by diminishing the ability of cancer cells to migrate in vitro which may translate to fewer bone metastases in vivo [65]. Other studies have suggested IB-MECA may have less myelotoxicity compared to other forms of chemotherapy, because it can induce proliferation of murine bone marrow cells via granulocyte-colony stimulating factor (GCSF) [66].

Regulation of osteoblast differentiation and function by adenosine

Repair of the skeletal system depends on the differentiation of osteoblasts from their progenitors (mesenchymal stem cells, MSCs) which have a broad, multilineage differentiation potential and express A1, A2A, A2B, and A3 receptors [67, 68] (see Fig. 3).

P1 receptors: A1R

Few studies have investigated the role of A1R in osteoblast development, though this receptor does have an apparent role in adipogenesis and adipocyte function [69]. Expression of A1R mRNA in rat MSCs is initially low but increases during differentiation to adipocytes by more than 800-fold. When

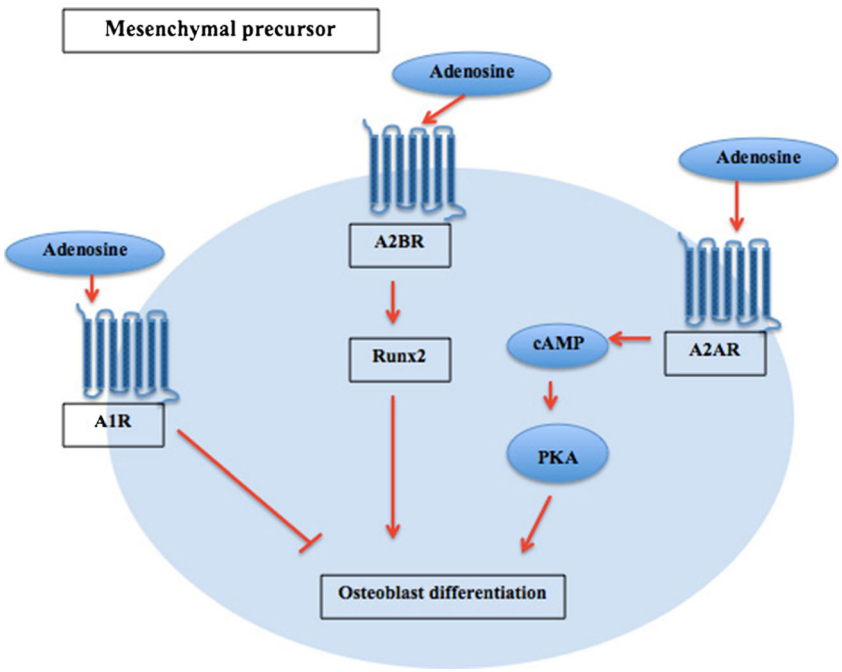

Fig. 3 Role of adenosine receptors in osteoblast differentiation. A1R activation inhibits osteoblast differentiation via the cAMP/PKA pathway, while A2AR enhances osteoblast differentiation through a cAMP/PKA-dependent mechanism. A2BR increases osteoblast differentiation and upregulates osteoblast-related genes including Runx2. Little is known about how A3R affects osteoblast differentiation

MSCs differentiate to osteoblasts, A1R mRNA expression increases less than fivefold and there is no change in protein expression [70]. Furthermore, A1R knockout mice do not have obvious changes in bone formation or osteoblast morphology, which suggests this receptor may not play a prominent role in osteoblast function [54]. However, in 2013, D'Alimonte et al. [71] showed A1R may favor the commitment of MSCs toward osteogenesis and produce an effect similar to what had already been proven for the A2B receptor. This group used MSCs isolated from human dental pulp stem cells (DPSCs), which can differentiate into osteoblasts in vitro and in vivo. They noted CCPA (A1R agonist) caused a dose-dependent increase in alkaline phosphatase (ALP) activity, enhanced extracellular matrix mineralization, and augmented runt-related transcription factor 2 (Runx2) expression, a key transcription factor in osteogenesis. They concluded A1R affected the mineralization potential of DPSCs in vitro. It appeared that this CCPA induced increase in osteogenesis was coupled to the canonical wingless (Wnt) signaling pathway, given that elevated levels of factors in this pathway were observed. When components of the Wnt pathway were silenced with DKK-1, there was no longer an increase in ALP and the differentiation of DPSCs was reduced [72]. Yet, other studies, have proposed the activation of Wnt may inhibit MSC differentiation [73]. The authors reconcile these conflicting results by suggesting that Wnt signaling may sustain cell differentiation when activated during the early part of this process and may deter differentiation when activated later on [72, 74].

P1 receptors: A2AR

Pulsed electromagnetic fields (PEMFs) stimulate osteoblast proliferation [75]. Vincenzi et al. found that treatment with PEMFs alone elicited a statistically significant increase in A2A mRNA levels, while PEMF exposure combined with CGS21680 had a synergistic effect on osteoblast proliferation [76]. In other studies A2AR stimulation has been shown to diminish inflammatory osteolysis and increase osteoblast numbers in inflamed bone [55]. In the context of a bone defect, A2AR helps restore bone homeostasis by increasing osteoblasts while decreasing osteoclast number and activity [77]. Yet, He et al. observed no effect on human osteoblast differentiation and mineralization with activation or downregulation of the A2AR [78]. Thus, the role of this receptor in osteoblast biology remains ambiguous.

Interestingly, activation of A2AR may have therapeutic potential in promoting bone regeneration. Mediero et al. observed enhanced bone regeneration, and an increase in bone volume and osteoblast expression of markers for bone formation including osteocalcin and osteonectin in mouse calvaria treated with either an A2AR agonist (CGS21680) or dipyridamole which blocks adenosine uptake. In addition, osteoclast markers were decreased in vitro and osteoclast numbers were reduced in vivo [77]. Adenosine receptor agonists or agents that increase local adenosine concentration may be preferred approaches to inducing bone regeneration following orthopedic surgeries compared to the currently used recombinant bone morphogenetic proteins, which are known to inflame nearby tissues, stimulate aberrant bone formation and carry higher complication rates [79].

P1 receptors: A2BR

Adenosine promotes osteoblast differentiation primarily through the A2BR receptor [68, 78, 80]. Rao et al. showed that A2B receptors may be integral in the maturation of bone tissue, as their expression increases as a function of time [81]. A2BR knockout mice have MSCs with decreased osteogenic potential in addition to lower bone density and delayed fracture repair [68]. Trincalvelli et al. used an allosteric modulator of A2BR, KI-7, and found this molecule-enhanced MSC differentiation to osteoblasts by increasing expression of osteoblast genes and accelerating osteoblast mineralization [82]. In contrast, blockade of A2BR with PsV603 diminished osteoblast differentiation of human MSCs [80]. Similarly, Takedachi et al. proposed that CD73 promotes osteoblast development through A2BR but not A2AR. Using MC3T3-E1 cells that overexpressed CD73, these researchers showed that treatment with an A2BR antagonist suppressed bone sialoprotein and osteocalcin levels, while this effect was not observed with an A2AR antagonists [27].

Another mechanism by which A2BR activation affects osteogenic differentiation is by increasing the expression of Runx2 which determines development of osteoblast versus chrondocyte lineages. Upregulation of Runx2 induces osteoblastic differentiation, while constitutive expression of the transcription factor in chondrocytes causes premature maturation and mineralization [68]. A2BR activation may also mediate MSC differentiation by modulating IL-6 levels. In the first stage of differentiation, A2BR reduces IL-6 which favors MSC commitment to osteoblasts, but then, in the terminal phase, this receptor causes release of IL-6 which then fosters survival of differentiated MSCs. In terms of therapeutic potential, IL-6 restoration by A2BR agonists could promote osteoblast viability and be used in tissue repair and regeneration [82]. Taken together, these results support the role of A2BR in osteoblast differentiation, but the downstream signaling following A2BR activation remains unclear. However, it is known that A2BR signaling is coupled to the activation of cyclic AMP through a Gs protein [83], and this is supported by Hsiae et al. who engineered a GPCR with a constitutively active Gs signal and found this dramatically enhanced bone mass [84]. In contrast, a

Table 1 Summary of effects of P1 receptors on osteoclasts, osteoblasts, and chondrocytes

	A1R	A2AR	A2BR	A3R
Osteoclasts	•Needed for osteoclast differentiation and function [52-54]	•Inhibits osteoclast differentiation and function [55]	•May inhibit RANKL-induced osteoclast formation [53]	•Decreases the number of osteoclasts, down regulates P13K, NFkB, and RANKL [64]
	•Promotes RANKL-induced NFkB activation, formation of TRAF6-TAK1 complex [53]	•Reduces bone pitting and loss in a model of wear particle-induced bone resorption [62]		
	•Blockade leads to increased bone density [51, 53, 54]			
Osteoblasts	•Unclear role, no morphologic changes in A1R knockout mice [54, 70]	•Enhances bone regeneration [77]	•Increases osteoblast differentiation and mineralization [82]	•Expressed on osteoblasts and osteoblast precursors, may increase osteoblast proliferation [21, 80]
	•May favor commitment of MSCs toward osteogenesis, enhance Runx 2 levels [71]	•Increases cell viability and cell number [76]	•Increases expression of Run × 2, sialoprotein, osteocalcin [68]	
	•Signals via canonical Wnt pathway [72]		•Blockade causes delayed fracture healing [68]	
Chondrocytes	•Not expressed [93]	•Reduces levels of inflammatory cytokines and joint destruction in collagen-induced arthritis [94, 95]	•Role not well understood	•Not expressed [93]
		•Agonist may prevent bone and cartilage erosion in RA [96]		

recent study by Hajjawi et al. demonstrated osteoblast numbers are unaffected by supraphysiological concentrations of adenosine or an A2B agonist (BAY606583). Differences in culture methods may contribute to this finding [85].

P1 receptors: A3R

There is little known about the role of A3R in osteoblast differentiation, though it is known to be expressed on osteoblast precursors and osteoblasts [21]. Use of the A3 agonist IB-MECA has not been shown to have an effect on osteoblast cell differentiation measured in terms of ALP activity. However, this receptor may have a small effect on cell proliferation [80].

Adenosine and cartilage metabolism

In normal articular cartilage, chondrocytes maintain matrix integrity to provide low-friction, painless joint movement [86, 87]. Chondrocytes communicate with each other via diffusible signals rather than direct cell-to-cell contact, and are capable of adenosine release in response to various physiological stimuli [88]. Healthy cartilage requires tight regulation of extracellular adenosine levels; if the levels become depleted, increases in glycosaminoglycan (GAG) release, production of matrix metalloproteinases (MMP-3, MMP-13), prostaglandin E2 and nitric oxide (NO) can lead to increased inflammation and may trigger cell death [89].

Endogenous adenosine is an important component of the feedback loop that limits cartilage damage during inflammatory processes, and when this homeostasis is not maintained joint destruction occurs [90]. Increased levels of ADA1 and ADA2, the enzymes that irreversibly convert adenosine to inosine, have been documented in the synovial fluid and synovial fibroblasts of patients with RA and systemic lupus erythematous [91, 92]. In fact, ADA levels correlate with disease severity in RA [93]. Treatment of chondrocytes with iodotubercidin (ITU), an adenosine kinase inhibitor, leads to higher levels of extracellular adenosine and reduced levels of PGE2 and NO release [88]. The use of ITU also reduces GAG release, which is a marker of proteoglycan degradation and cartilage damage. Local levels of adenosine appear to

modulate synovial inflammation, which is supported by the observation that extracellular adenosine has immediate anti-inflammatory benefits including reduction of PGE2 and NO release [93].

P1 receptors: A2AR

Though P1 receptors are expressed on chondrocytes A1 and A3 receptors are not present [89, 94]. Application of the A2A-specific agonist DPMA, increases cAMP accumulation in equine chondrocytes which further supports the presence and function of A2A receptors on these cells [88]. Stimulation of this receptor using polydeoxyribonucleotide (PDRN), diminishes production of TNF-alpha, macrophage inflammatory protein 1-alpha, and prevents development of collagen-induced arthritis in mice. Also, human chondrocytes cultured with PDRN exhibit higher levels of IL-10, an anti-inflammatory cytokine that reduces proinflammatory cytokines, and production of MMPs [95]. Treatment of murine chondrocytes previously stimulated with IL-1β, with CGS-21680 (A2AR agonist) appears to reduce TNF-alpha, IL-6, MMP-13, and inducible nitric oxide synthase activity [96]. Application of CGS-21680 to mice with collagen-induced arthritis reduces paw edema, and histological alterations of the joint suggesting an A2AR agonist may help prevent bone and cartilage erosion in RA [97]. Therefore, there may be a role for activating this receptor to reduce the inflammatory joint destruction in RA.

Use of NECA, a nonselective adenosine receptor agonist in rat mesangial cells has been shown to upregulate the activity of 5′ nucleotidase (5NT), an adenosine-generating enzyme [98]. This enzyme modulates cartilage homeostasis and displays increased levels of activity in diseased cartilage [99, 100]. Chondrocytes in osteoarthritic lesions show elevated 5NT activity relative to non-lesioned areas, and this causes increased extracellular adenosine levels, and in turn, higher intracellular concentrations via transporters. Studies in Mc625 chondrocytes demonstrate that incubation with adenosine and an ADA inhibitor (EHNA) reduces cell number while promoting DNA fragmentation, caspase 3-activation and PARP cleavage. This finding suggests that when adenosine concentrations increase within the chondrocyte and degradation is blocked with EHNA, apoptosis can occur. Together, these findings imply chondrocyte death in a mouse model of OA may be driven by increased adenosine production through 5′NT [89]. Therefore, adenosine may play a deleterious role in osteoarthritis although the protective effects of adenosine for chondrocytes discussed earlier suggest the need for further research.

Conclusion

Multiple lines of evidence point toward adenosine and adenine nucleotides as potent regulators of bone metabolism. P1 receptors may constitute novel targets for therapeutic intervention in bone disease. Adenosine A2A and A2B receptor stimuli, both direct and indirect, can promote bone regeneration and bone healing and stimulation of A2A receptors can block the development of inflammatory bone destruction, a common problem in patients who have undergone prosthetic joint replacement (see Table 1). Further research will be required to develop effective purinergic therapies to restore and maintain bone homeostasis.

Abbreviations

ADA adenosine deaminase, *ADP* adenosine diphosphate, *AMP* adenosine monophosphate, *ATP* adenosine triphosphate, *CD39* ectonucleoside triphosphate diphosphohydrolase 1, *CD73* ecto-5′nucleotidase, *DPSCs* dental pulp stem cells, *ENT1* equilibrative nucleoside transporter 1, *ERK* extracellular signal-regulated kinases, *FGF23* fibroblast growth factor 23, *GCSF* granulocyte-colony stimulating factor, *IL10* interleukin 10, *IL-1β* interleukin 1 beta, *IL-6* interleukin 6, *MAPK* mitogen-activated protein kinase, *M-CSF* monocyte colony stimulating factor, *MMP-13* matrix metallopeptidase-13, *MSC* mesenchymal stem cells, *MTX* methotrexate, *NFkB* nuclear factor kappa-B, *NPP-1* nucleotide pyrophosphatase phosphodiesterase 1, *OPG* osteoprotegerin, *PEMFs* pulsed electromagnetic field stimulation, *PI3K* phosphoinositide 3-kinase, *PKA* protein kinase A, *RANK* receptor activator of NFkB, *RANKL* receptor activator of NFkB ligand, *Runx2* runt-related transcription factor 2, *TNAP* tissue non-specific alkaline phosphatase, *TNF-α* tumor necrosis factor alpha, *TRAF6* TNF receptor associated factor 6, *TRAP* tartrate-resistant acid phosphatase, *Wnt* wingless-type integration site family.

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Compliance with ethical standards This article does not contain any studies with human participants or animals performed by any of the authors.

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