




Adult mesenchymal stem cells: is there a role for purine receptors in their osteogenic differentiation?

Marzia Carluccio^{1,2,3} · Sihana Ziberi^{1,2,3} · Mariachiara Zuccarini^{1,2} · Patricia Giuliani^{1,2} · Francesco Caciagli² · Patrizia Di Iorio^{1,2} · Renata Ciccarelli^{1,2,3} 

Received: 22 November 2019 / Accepted: 4 May 2020 / Published online: 4 June 2020
© Springer Nature B.V. 2020

Abstract

The role played by mesenchymal stem cells (MSCs) in contributing to adult tissue homeostasis and damage repair thanks to their differentiation capabilities has raised a great interest, mainly in bone regenerative medicine. The growth/function of these undifferentiated cells of mesodermal origin, located in specialized structures (niches) of differentiated organs is influenced by substances present in this microenvironment. Among them, ancestral and ubiquitous molecules such as adenine-based purines, i.e., ATP and adenosine, may be included. Notably, extracellular purine concentrations greatly increase during tissue injury; thus, MSCs are exposed to effects mediated by these agents interacting with their own receptors when they act/migrate in vivo or are transplanted into a damaged tissue. Here, we reported that ATP modulates MSC osteogenic differentiation via different P2Y and P2X receptors, but data are often inconclusive/contradictory so that the ATP receptor importance for MSC physiology/differentiation into osteoblasts is yet undetermined. An exception is represented by P2X7 receptors, whose expression was shown at various differentiation stages of bone cells resulting essential for differentiation/survival of both osteoclasts and osteoblasts. As well, adenosine, usually derived from extracellular ATP metabolism, can promote osteogenesis, likely via A2B receptors, even though findings from human MSCs should be implemented and confirmed in preclinical models. Therefore, although many data have revealed possible effects caused by extracellular purines in bone healing/remodeling, further studies, hopefully performed in in vivo models, are necessary to identify defined roles for these compounds in favoring/increasing the pro-osteogenic properties of MSCs and thereby their usefulness in bone regenerative medicine.

Keywords Mesenchymal stem cells/multipotent stromal cells · Bone regenerative medicine · Osteogenic differentiation · Bone repair · Purine receptors

Introduction

Bone is a dynamic tissue undergoing a continuous remodeling to respond to the needs of the body during growth and adult life. While upon fractures bone is repaired by healing without

formation of scar tissue, large innate osseous defects fail to heal [1]. Additionally, aging as well as immune/degenerative diseases can alter bone structure and, in these conditions, the local environment is often unfavorable to osteogenesis due to damage to the surrounding soft tissues and vasculature [2, 3].

✉ Renata Ciccarelli
renata.ciccarelli@unich.it

Marzia Carluccio
marzia.carluccio@unich.it

Sihana Ziberi
sihana.ziberi@unich.it

Mariachiara Zuccarini
mariachiara.zuccarini@unich.it

Patricia Giuliani
patricia.giuliani@unich.it

Francesco Caciagli
f.caciagli@unich.it

Patrizia Di Iorio
patrizia.diiorio@unich.it

¹ Department of Medical, Oral and Biotechnological Sciences, University of Chieti-Pescara, Via dei Vestini 29, 66100 Chieti, Italy

² Center for Advanced Studies and Technologies (CAST), University of Chieti-Pescara, Via L. Polacchi, 66100 Chieti, Italy

³ StemTeCh Group, Via L. Polacchi, 66100 Chieti, Italy

A new perspective in the cure of bone dysfunctions is offered by the use mesenchymal stem cells (MSCs), which show good self-renewal and differentiation potential, in particular toward osteogenesis. Therefore, these cells are intensely studied, representing a possible new therapeutic tool in bone regenerative medicine. The acronym “MSCs” has also been used to indicate the same cells as multipotent stromal cells [4, 5] and, more recently, as medicinal signaling cells, to emphasize their property to secrete bioactive factors with trophic/regenerative properties at the sites of injury [6]. Accordingly, preclinical and clinical studies demonstrated that the differentiation capabilities of MSCs can be modulated by endogenous substances, even produced by the same cells, or related drugs. Among these, we focused on purines, which are ancestral molecules synthesized and released from all cells, including MSCs that are provided with a large variety of membrane receptors responsive to purines [7]. Noteworthy, the extracellular concentration of these compounds greatly increases during tissue injury. Thereby, MSCs are likely exposed to them when they migrate in vivo or are transplanted into a damaged tissue. Thus, there is a growing interest to explore how endogenous/exogenous purines influence MSC properties by interacting with their own receptors.

Based on these premises, the aim of this review is to summarize the principal events occurring during osteogenesis and bone repair, focusing on the role played by MSCs and purines in these processes. Several excellent reviews have addressed similar topics over the past 5 years [8–11]. Here, we tried to highlight the positive data obtained so far, but also some limitations related to the research on the use of MSCs and of purine compounds in regenerative bone medicine.

MSC involvement in osteogenesis and bone repair

Bone components

Bone is a heterogeneous tissue formed by two components in close relationship, which are as follows:

- the extracellular matrix (ECM), composed of a mineral phase (mainly hydroxyapatite crystals, 50–70%) and an organic phase (20–40%), constituted by collagen type I fibers and non-collagenous proteins including growth factors (GFs), cytokines, and proteoglycans [12];
- the bone cells including:
 - *osteoblasts*, the bone-forming cells deriving from MSCs [13];
 - *osteocytes*, the most abundant cells in adult bone [14], in turn deriving from terminally differentiated osteoblasts, which, contrary to osteoblasts, can survive throughout the life of an individual [15] and play critical roles in

the regulation of differentiation and function of osteoblasts and osteoclasts [16];

- *osteoclasts* generated in the bone marrow from mononuclear monocyte-macrophage precursors derived from the hematopoietic lineage, which resorb bone, thereafter undergoing apoptosis [17]

Continuous bone remodeling, which allows the skeleton to grow, adapt, and repair itself, is due to a constant balance between formation and resorption processes carried out by osteoblasts and osteoclasts, respectively.

MSCs and osteogenesis

Osteogenesis is the process of development and formation of bone tissue. Undifferentiated MSCs, present in the bone matrix, start to differentiate into mesenchymal osteoblasts (MOBL), which secrete collagen throughout the matrix, forming a woven structure, and into surface osteoblasts (SOBL), which secrete collagen fibrils creating the highly oriented lamellar bone. Once this process finishes, osteoblasts mature into osteocytes surrounded by collagen matrix [18]. MSCs likely receive biochemical stimuli from neighboring cells able to influence their differentiation into bone precursors. About this, in vitro co-cultures of MSCs with osteocytes show a greater osteogenic differentiation than those with osteoblasts [19].

Multiple molecular pathways play important roles in regulating MSC proliferation, differentiation, and apoptosis in bone. In particular:

- Wnt signaling is recognized as a key regulator of bone homeostasis and remodeling so that gene mutation of factors in this pathway may lead to abnormal bone formation [20]. Accordingly, β -catenin inactivation in MSCs can inhibit osteogenic differentiation [21–23];
- the adenylate cyclase/cAMP system also affects osteogenesis in human—but not in rodent-derived MSCs; in particular, increase in cAMP levels can either stimulate or inhibit osteogenesis in human MSCs, depending on the duration, rather than the strength of the signal [24, 25];
- the activation of extracellular-regulated kinases 1 and 2 (ERK1/2) has also been demonstrated by studies in bone marrow-derived MSCs (BMSCs) committed toward osteoblast differentiation in response to various mechanical stimuli [26, 27].

MSC osteogenic differentiation is also regulated by several transcription factors, including Runx-2 (Runt-related transcription factor 2) [28], Osterix (Osx) [29], Mouse segment homeobox 1/2 (Msx1/2) [30], and T cell factor/lymphoid enhancer factor (TCF/LEF) [31], which are

interdependent and closely linked with each other to form a network in the mentioned signaling pathways.

The MSC commitment toward an osteogenic phenotype has long been studied *in vitro*. This process is induced by the presence of dexamethasone, ascorbic acid, and β -glycerol phosphate in the culture medium [32] and has been divided into three stages [33] along which MSCs express markers typically present in bone forming osteoblasts [34, 35] (see Fig. 1).

MSCs and bone regeneration

The intrinsic bone regeneration capacity plays a substantial role in the repair process in response to injury [36]. Periosteal progenitors are the primary cell source that gives rise directly to fracture callus, whereas bone marrow MSCs (BMSCs), which seem to be fairly abundant in normal and pathological bone *in vivo* [37], are crucial in supporting healing through paracrine secretion of trophic and immunomodulatory factors [38]. However, in bone diseases such as osteoporosis and non-union, the regeneration process fails. Thus, different strategies are used to improve the impaired or “insufficient” bone repair, including autologous bone graft, considered to be the gold standard procedure, free fibula vascularized graft, allograft implantation, and use of GFs, osteoconductive scaffolds, distraction osteogenesis and, also, injection/use of osteoprogenitor cells [39]. The latter application is supported by a large number of scientific articles, mostly reported in the following chapters.

The first studies on MSC properties and characteristics were carried out on pluripotent cells derived from embryos in the earliest stages of maturation (blastocysts). However, the use of these pluripotent cells raised numerous ethical issues precluding subsequent applications for clinical purposes. Thus, investigations were directed toward adult somatic cells induced to become pluripotent stem cells (iPS) [40]. Unfortunately, both embryonic and iPS cells showed the worrying ability to form teratomas and resulted to be tumorigenic [41, 42], which was an insurmountable obstacle to proceed in investigating their clinical applicability. In the early seventies, after the seminal paper by Friedenstein et al. [43] demonstrating that in bone marrow there are resident cells with stemness features, many other papers pointed out that this kind of cells, characterized as MSCs, are present in almost all adult tissues and are responsible for cellular regeneration and tissue homeostasis. The most important advantages in the potential use of these cells in comparison with embryonic or iPS cells are that they do not rise ethical issues, show no evident antigenicity when used for allogenic transplantation, and have a very low tumorigenic potential, even though some reports indicated that a risk exists [44, 45].

The current approach for delivering osteogenic cells in humans directly to the regeneration site includes use of bone-marrow aspirate from the iliac crest, which also contains GFs [46]. However, the concentration and quality of MSCs may vary significantly, depending on the individual (especially in older people), the aspiration sites, and techniques used. Thus, alternative sources of MSCs are under extensive research.

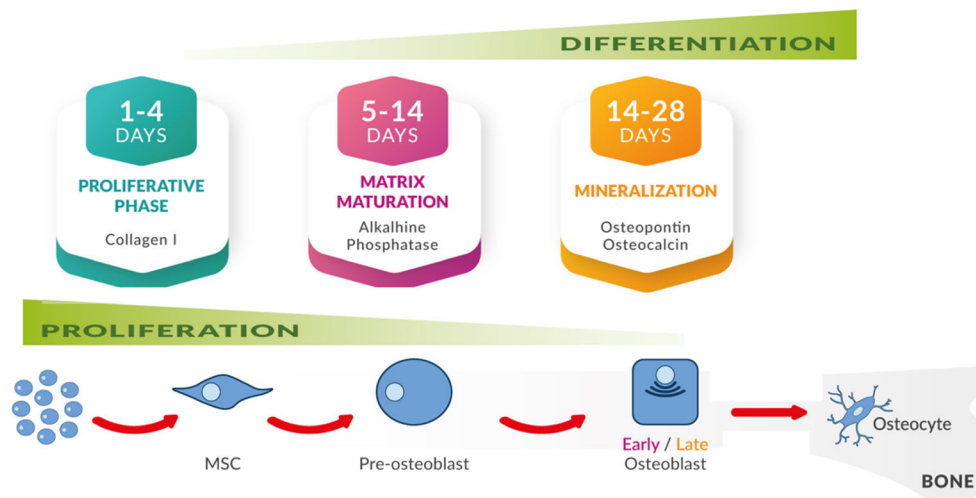


Fig. 1 Steps in the osteogenic differentiation of MSCs: MSCs actively proliferate during the initial stages of osteogenesis and produce collagen [34]. This is followed by early cell differentiation (days from 5 to 14), during which MSCs show a spindle-shaped aspect and decreased proliferation rate, while they start expressing osteogenic markers such as alkaline phosphatase (ALP) secreted by early osteoblasts (matrix maturation phase). After an initial peak, ALP level tends to decline and

in the final stage (days from 14 to 28) a high expression of osteocalcin and osteopontin, secreted by late osteoblasts, is observed, followed by calcium and phosphate deposition (mineralization phase) [33, 35]. At the end of the bone forming phase, osteoblasts become osteocytes, the most abundant cells in adult bone, with a small cell body and numerous long, dendritic-like cytoplasmic prolongations forming a canalicular system inside bone [14], or bone lining cells (not shown)

Availability of MSCs from different sources

MSCs are located in specialized niches of differentiated organs and are deputed to maintain tissue homeostasis [47]. Accordingly, they can be isolated from diverse human adult tissues (i.e., bone marrow, adipose, or dental tissues), but clearly also from embryonic annexes (umbilical cord, placenta) or fluids (amniotic liquid). Even in the adult brain, there are specific zones, mainly the subventricular one, in which MSCs are present, assuring a renewal of neural cells, although in a limited manner, in physiological and pathological conditions [48, 49].

Regardless of their tissue origin, MSCs can differentiate into cells of the mesenchymal lineage, which includes adipocytes, osteoblasts, chondrocytes, tenocytes, skeletal myocytes, and cells of the visceral mesoderm [50, 51], but also into cells of ectodermal and endodermal origin, such as hepatocytes [52], neurons [53], and cardiomyocytes [54, 55]. However, MSC differentiation potential has mainly been investigated toward cells of the mesenchymal lineage [56].

Although in 2006, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy defined four criteria as follows: (i) ability to adhere to plastics when placed under standard culture conditions and (ii) to differentiate into osteoblasts, chondrocytes, and adipocytes; (iii) presence of CD73, CD90, and CD105 markers, and (iv) absence of hematopoietic markers CD14, CD11b, CD34, CD45, CD19, and CD79 α) as needed to classify/define a cell as MSC [57], the phenotypic characterization of MSCs still remains a field of investigation due to the lack of unique distinctive markers for their analysis and isolation. From literature it is evident that (i) isolation of MSCs from easily accessible sources and with a great potential of cell expansion and multi-lineage differentiation remains the ideal goal; (ii) MSCs from bone marrow and adipose tissue are the most widely studied and being tested in *in vitro* and *in vivo* studies of regenerative medicine and cell-based therapy. In the paragraphs reported below, we summarized data on MSCs isolated from different tissues/fluids.

MSCs from bone marrow

BMSCs, in addition to being the first ones to be investigated as multipotent cells to be directed toward osteogenic differentiation, are considered the reference cell type with which MSCs deriving from other sources are compared. One limiting factor for their use is, in general, their availability, since they are present at low rate, which further decreases with aging [58, 59]. Moreover, cell collection requires an invasive procedure associated with pain and morbidity.

MSCs from neonatal tissues and amniotic fluid

Neonatal tissues such as umbilical cord, placenta, and amniotic membrane are other sources of MSCs with high proliferation rates and potent differentiation capacities, as evaluated in preclinical models [60, 61]. Additionally, human term amniotic fluid could be an ideal source of MSCs. Indeed, preclinical studies recently demonstrated the following: (i) the feasibility of collecting significant volumes of this fluid, otherwise discarded, from a wide number of potential donors; (ii) the high yield of MSCs obtainable; (iii) the properties of these MSCs, given their neonatal origin; and (iv) their suitability to be reprogrammed into the pluripotent state. Thus, these MSCs are a promising and plentiful resource for further evaluation in bio-banking, cell therapy, disease modeling, and regenerative medicine applications [62, 63].

MSCs from dental tissues

MSCs from dental tissues (DSCs) also show self-renewal capacity and multi-differentiation potential. So far, eight main DSCs have caught the interest of researchers, namely dental pulp stem cells (DPSCs), which were the first to be isolated and studied, stem cells from human exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), dental follicle progenitor cells (DFPCs), alveolar bone-derived MSCs (ABMSCs), stem cells from apical papilla (SCAP), tooth germ progenitor cells (TGPCs), and gingival MSCs (GMSCs) [64]. Among them, DPSCs and SHED have been more extensively investigated as autologous stem cells to use for regenerative purposes and tissue engineering therapies (reviewed by [65]).

MSCs from adipose tissue

MSCs can be isolated from subcutaneous (SAT) and visceral (VAT) adipose tissue [66]. Of particular interest is SAT that consists mature adipocytes and a heterogeneous cell population, called stromal vascular fraction (SVF), comprising fibroblasts, pericytes, pre-adipocytes, endothelial cells, blood cells, and adipose stem cells (ASCs) [67]. Noteworthy, ASCs shows easy accessibility and can be harvested also via liposuction under local anesthesia, which is a painless and reasonably comfortable way for patients to provide a good amount of autologous stem cells [68]. Moreover, they are more abundant and provided with higher proliferation rate than BMSCs, although both cell types have a similar multi-lineage differentiation potential [66, 68–72]. Interestingly, the ASC secretome includes active molecules such as numerous GFs [73]. Thus, ASCs could be ideal cells in regenerative medicine [74, 75]. However, there are still some barriers to overcome: (i) a precisely defined source population of ASCs, (ii) isolation procedures according to regulatory clinical standards, (iii) better

understanding of the behavior of ASCs in their niche and of their interaction with scaffolds, (iv) evaluation of their safety and efficacy in clinical applications, and (v) long-term follow-up studies on the consequences in patients [73, 75, 76].

Preclinical and clinical applications of MSCs in bone repair

Preclinical studies

Even though undifferentiated MSCs, left in a normal culture medium for a long period (> 30 days), spontaneously differentiate into osteoblasts [77], compelling evidence showed the MSCs are committed toward osteogenesis under suitable culture conditions [78, 79]. The use of this model allowed to characterize the principal stemness features of MSCs from various sources, their differentiation potential, and distinctive properties, such as their immune-modulatory effect, the ability to provide trophic support to other cells, to slow down degenerative processes, to secrete biochemical substances (GFs, cytokines, neurotransmitters, and extracellular matrix compounds), and to recruit endogenous stem/precursor cells to defective sites for new tissue regeneration/repair [80]. Additionally, it has also been demonstrated that MSC osteogenic differentiation can be supported by suitable biomaterial properties (i.e., adhesivity, stiffness, nanocrystalline structure, and degradability) [81] and improved by the associate use of specific GFs and selected scaffolds [82].

Fundamental preclinical evidence on the possible MSC therapeutic applications to treat human diseases has also been achieved by using rodents or large animal experimental models, including companion (dogs, cats, and horses) and farm animals (cows, sheep, goats, and pigs). These studies pointed out the improvement obtained by the MSC use in bone healing and outcome from fracture or segmental defect repair [83, 84] as well as in congenital defects such as spina bifida [85, 86] and chest wall defects [87]. A particular MSC application has been done in experimental dentistry to regenerate oral/dental tissues [88–90] and to cure inflamed periodontium [91, 92]. These models represent an important translational paradigm that closely mimics the clinical environment of humans [93].

Unfortunately, although articles on MSC-based bone reconstruction techniques are numerous, there is a multitude of experimental protocols leading to a wide range of parameters and data, so that it is still difficult to achieve significant conclusions as to the “most predictable” model in stem cell reconstruction [94]. Consequently, there is need for additional studies with more homogenous experimental designs and data analysis in order to gain useful advances in the use of MSCs for bone reconstruction.

Clinical studies

As above reported, in complicated fractures, tumor resections, spinal arthodesis, or large bone defects, healing process may fail. In these cases, clinicians have adopted different therapeutic strategies [95, 96], but clinical trials including MSC use mostly concerns transplantation of human autologous cells, alone or in combination with scaffolds to treat different bone diseases [97–103]. Similar techniques favor spinal fusion following spinal arthrodesis performed in the treatment of spinal trauma, degenerative diseases, tumors, or infections [84]. Additionally, phase I/II clinical studies performed on patients with spinal cord injury (SCI) at the cervical or thoracic level, showed that (i) injection of BMSCs had significant regenerative effects within a therapeutic window of 10–30 days after SCI [104, 105] and (ii) autologous MSC transplantation may be a safe procedure, leading to improvements in motor-sensory functions and tissue regeneration in almost half of patients, thus enhancing their quality of life [106].

The clinical effectiveness of stem cell therapies has also been evaluated in dentistry, mainly for alveolar ridge augmentation preceding the insertion of dental implants, in which BMSCs from the iliac crest have been the most well characterized and commonly used for their osteogenic ability [107], whereas periosteum-derived stem/osteoprogenitor cells, ASCs, and DSCs have been applied for orofacial bone regeneration [108, 109]. Finally, the possible successful MSC applications in pediatric diseases such as osteogenesis imperfecta, juvenile idiopathic arthritis, simple bone cysts, and osteonecrosis of the femoral head have been reported [110], representing an encouraging premise for MSC application to treat incurably ill pediatric patients. As for ASCs, their clinical application ranges from various areas of regenerative medicine, such as hair [111] or peripheral nerve regeneration [112], cardiovascular [113], pancreatic [114] or hepatic repair [115], plastic surgery, and esthetic medicine [75], to treatment of chronic limb defects and diabetic wounds [116, 117]. Additionally, intra- or periarticular ASC injection can support cartilage regeneration in patients suffering from osteoarthritis, chondromalacia, osteonecrosis of the femoral head, or a meniscus tear. In contrast, use of ASCs in bone tissue engineering is still entirely experimental [118] while suggesting its potential benefits in various orthopedic applications [119]. Noteworthy, the ASC paracrine secretion of GFs and the co-culture of these cells with endothelial cells (ECs) may accelerate their phenotypic maturation into ECs [120], thus making them suitable for vascular prosthesis seeding.

In conclusion, numerous clinical studies demonstrated the potential use of human autologous MSCs for bone tissue engineering strategies. However, the major part of them emphasizes that further studies are still needed to improve cell-based therapy in bone regeneration and to decipher the biological and molecular mechanisms that are involved.

It is also to emphasize that a new research avenue has been opened concerning the use of MSC-derived conditioned medium (CM) that is rich of factors able to increase tissue synthetic activity. A recent review [121] reported that a discrete number of *in vitro* and *in vivo* investigations has already been performed using MSC-derived CM in bone and periodontal defects, and one study, carried out in patients needing alveolar bone regeneration, showed that MSC-derived CM caused bone formation without systemic or local complications. This obviously means that the identification/characterization of the factors involved in the promotion of the osteogenic differentiation and bone repair becomes extremely important.

Purines and MSC osteogenic differentiation

In bone regeneration, the significance and the experimental therapeutic use of MSCs is ever more investigated. As well, the involvement of local signals during fracture healing and repair has been demonstrated by various studies, which highlighted the role of GFs and related signaling pathways during this process [122]. Thus, it is important to consider that MSCs, if used for bone regenerative medicine, will likely meet an environment rich of GFs and also of other kinds of substances released from cells during tissue injury that could influence MSC ability to support bone repair. Among these agents, we focused on purines.

A brief overview on purines

Many excellent reviews have given a comprehensive picture of the significance of purines inside the cells, the various mechanisms of their release from cells into the extracellular fluid, the carriers involved in the recovery and sometimes also in the release of nucleosides and nucleobases, and the enzymes deputed to their interconversion or degradation (for a review see ref. [123]). Therefore, here we reported a brief introductory overview of the main general findings on purines to then deepen some aspects about them in MSCs.

Purines together with pyrimidines are ancestral and ubiquitous molecules present in animal and plant cells as constituents of nucleic acids or participants in biochemical reactions and energy transport inside the cell [124, 125]. Moreover, agents such as cAMP and cGMP act as important second messengers at intracellular level during signal transduction, whereas guanine-based purines behave as modulators of intracellular processes, especially in relation to the G protein activity in signal transduction [126]. However, purines and some pyrimidine compounds (UTP, UDP, and UDP-glucose) also act at extracellular level, being the release of endogenous nucleotides into the extracellular space a critical step for the initiation of purinergic signaling events. Nonlytic release of ATP, UTP, UDP-glucose, and other nucleotides occurs in all

cell types and tissues via both constitutive mechanisms, that is in the absence of external stimuli and, to a greater extent, as a response to biochemical or mechanical/physical stimuli [127]. In particular, ATP and ADP and also uridine-based nucleotides are released from different cell types by cargo-vesicle trafficking and secretory granule exocytosis [9, 128]. The physiological release of adenosine has also been reported in some cell types, including neuronal cells, kidney cells, cardiomyocytes, and immune cells [129], whereas in particular stress conditions, adenosine can be released into the extracellular milieu by ubiquitously expressed transporters, which, however, are mostly deputed to uptake adenosine inside the cells [130]. Finally, all endogenous nucleotides and nucleosides can be discharged into the extracellular space due to different cell stressors causing damage to the plasma membrane [128, 129, 131]. Thus, ATP efflux can occur under various more or less noxious stimuli, including shear stress, hypotonic swelling, stretching, hydrostatic pressure, hypoxia/hypoglycemia/ischemia, and the consequent cellular energy deprivation, as well as in response to Ca^{2+} agonists [128]. Even though less known, also GTP and related compounds are found in the pericellular fluid and behave as extracellular modulators affecting the glutamatergic activity and exhibiting behavioral and trophic effects on neuronal cells [132].

The concentration of extracellular purines and pyrimidines is tightly controlled by multiple nucleotide and nucleoside-metabolizing enzymes with either hydrolyzing or transphosphorylating activities, which predominantly function as membrane-bound enzymes (ecto-enzymes) rather than as soluble or microvesicle-associated ones [10, 11, 133]. In addition, there is a system to remove nucleobases and also nucleosides from the extracellular fluid, represented by specific transport proteins usually functioning to take nucleotide derivatives up [134]. More in detail, two major classes of nucleoside transport systems have been described in mammalian cells, the equilibrative and the concentrative transport systems. The former mediates nucleoside transport in both directions, this depending on the nucleoside concentration gradient across the plasma membrane, while the latter is Na^+ -dependent and the movement of the nucleoside, regardless of its concentration gradient, is coupled to that of the sodium ion. However, nucleosides, nucleobases, and their analogs are also substrates for other carrier proteins such as organic anion and cation transporters and ABC transporter proteins that mostly function as efflux transporters.

Once released into the extracellular space, purines and pyrimidines activate different types of specific receptors, which are classified based on their molecular structure and agonist specificity as P1 receptors (P1R), selective for adenosine (A1, A2A, A2B, and A3 receptor subtypes) and coupled to G proteins [10, 135], and P2 receptors (P2R), mostly sensitive to ATP, in turn divided into eight metabotropic P2Y and seven ionotropic P2X receptors on the basis of mechanism of action,

pharmacology, and molecular cloning [136]. Of note, some P2Y receptors, namely P2Y₂, 4, 6, and 14, are mainly sensitive to uridine-derived nucleotides [137]. Each of these receptors is widely but differentially expressed in virtually all cell types [9, 136]. Noteworthy are as follows:

- Purine-metabolizing ecto-enzymes are responsible for the nucleotide signal termination and for maintaining the balance of purine concentration in the extracellular environment [10]. This sophisticated mechanism enables a fine-tuning regulation of purine levels and can be altered in pathological conditions [138];
- purinergic signaling is involved in the regulation of different processes such as cell proliferation and differentiation, chemotaxis, cytokine release, formation of reactive oxygen species, cell fusion, phagocytosis and induction of cell death for apoptosis or necrosis, inflammation, and cancer [135, 139].

An overall picture of purine turnover at intra- and extracellular levels is depicted in Fig. 2.

In the next paragraphs, we reported more detailed information about the purine system in MSCs. These data have mainly been desumed from primary and/or immortalized cell lines cultures, grown in basal conditions, and/or in classic medium favoring the osteogenic differentiation [32]. As for primary cell cultures, it is to underline that in most articles, the number of cell passages in vitro was hardly reported, even though it is known that use of some MSCs after the 6th–7th passage makes them less prone to differentiate [140]. Moreover, while the influence of purines on MSC osteogenic differentiation has been evaluated in MSCs deriving from different animal and human tissue sources, investigation on differences existing in the expression of purine receptors as well as in their responsiveness to purines ligands did not received similar attention and is still poor. Thus, the results are not always easily comparable (see in the next paragraphs).

Release of ATP during osteogenic differentiation and bone remodeling

In recent years, purines are emerged as key players also in the context of bone remodeling. Indeed, nucleotides are important local factors in the osteogenic differentiation of MSCs/osteoprogenitors, from which are constitutively released under basal and/or stressful conditions through the same mechanisms listed above for all cell types. Among them, exocytosis seems to be a major way for releasing ATP from osteoblasts [141–143], but other findings suggest that maxi-anion channels [144], connexins, namely connexin 43 [145], and the P2X7 receptors (P2X₇R) themselves through membrane pore opening [146] contribute to this event. Release mechanisms and total amounts of extracellular ATP are highly

dependent on cell differentiation stage, mature bone-forming cells releasing up to sevenfold more ATP than immature proliferating cells [143]. In particular, the expression of connexin 43, which is predominant in bone cells, increases with osteoblast differentiation, possibly allowing gap junction hemichannels to become prevalent and to exert an important functional role in bone cell mechano-transduction [147]. Noteworthy, extracellular nucleotide concentrations are low in basal condition, mainly due to the presence of ubiquitous ecto-nucleotidases (e-NTPDases) and other ecto-phosphatases. Consequently, purinergic signaling is influenced by multiple factors, including the activity of different receptor subtypes, nucleotide releasing pathways, and ecto-nucleotidases [10, 148]. However, the interplay between purinoceptors and ecto-enzymes is still largely unknown in bone cells, leading to a gap in our knowledge regarding as to how they are organized in the control of bone remodeling.

Additionally, many studies showed increments of released ATP in response to external stimuli, namely hypoxia [143] and mechanical stress [149]. In the case of hypoxia, this stimulus results in an increased ATP release from osteoblasts up to 2.5-fold without affecting cell viability, being the mechanism mainly driven by exocytosis [143]. Ultrasound stimulation, a known method to accelerate fracture healing, was also found to promote ATP release from osteoblasts [150]. These and many other studies reported below suggest that the release of nucleotides in response to different stimuli may represent a local trigger of osteogenesis in bone forming niches [151].

Expression and role of purine ecto-enzymes in MSC biological functions

Like virtually all cells, MSCs express different e-NTPDases on their membrane surface, which metabolize extracellular ATP and other nucleotides, thus contributing to modulate the purinergic receptor signals [10, 11]. This evidence has been reported in undifferentiated MSCs from mouse bone marrow and human umbilical cord blood [152, 153], in which these enzymes showed substantial differences as for their expression level, activity, and substrate specificity, likely depending on the tissue of origin and the biological MSC potential. For instance, the e-NTPDase activity is negligible in murine MSCs [154]. In contrast, ecto-5'-nucleotidase (e-5'NT), also known as CD73 and hydrolyzing AMP to adenosine, is highly expressed on the plasma membranes of undifferentiated MSCs from adult [155] and embryo tissues [152] and is considered one of the mesenchymal stem cell markers [57]. Since adenosine increases the proliferation rate of animal and human MSCs, likely by A_{2A} receptor activations [155, 156], it is conceivable that the high proliferative potential of these cells is supported by adenosine present in the extracellular environment thanks to the e-5'NT activity.

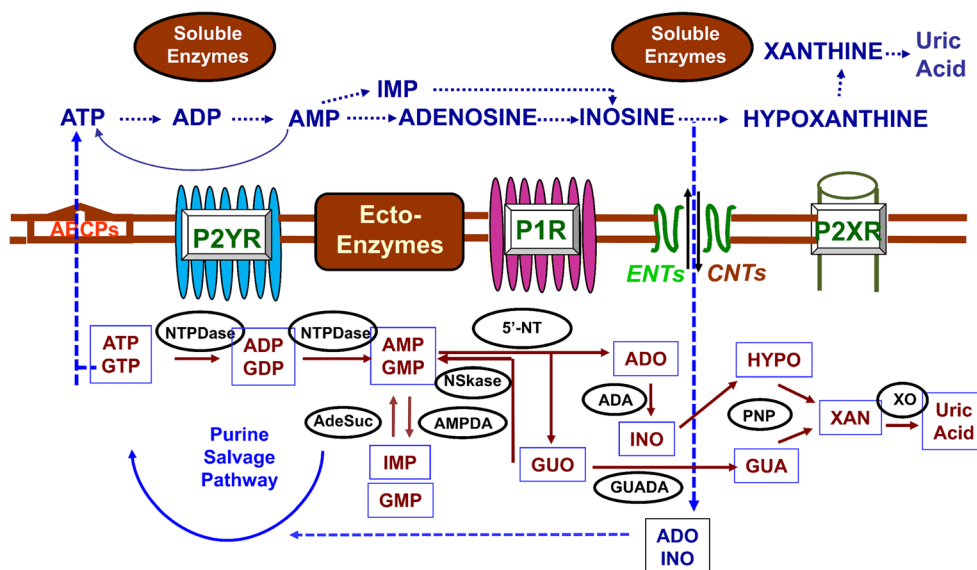


Fig.2 Synthesis, release, and metabolism of intra- and extra-cellular purines. Purine nucleotides are produced in cells by *de novo* synthesis and the salvage pathway, which recycles nucleosides and purine bases, also deriving from the turnover of nucleic acids, especially RNA [125]. Nucleotides (here, we consider mainly ATP) are released from virtually all cells in physiological conditions by different types of stimulation; additionally, cell damage and membrane leakage determine a huge purine increase in the extracellular fluid [127–129]. Besides secretory mechanisms typical of nervous cells, ATP release is mediated by connexin/pannexin hemichannels, facilitated diffusion by nucleotide-specific ATP-binding cassette transporters, and multiple organic anion transporters. Also, adenosine, in particular stress conditions, is released into the extracellular fluid by transporters; the principal function of which is, however, to bring adenosine back into the cells. The nucleoside transport system comprises equilibrative (ENTs) and concentrative transporters (CNTs) [131, 135] in mammalian cells. Thus, nucleosides are transported by ENTs in both directions depending on their concentration gradient across the plasma membrane, whereas the transport by CNTs is coupled to the concentration gradient of sodium

Interestingly, the purine enzyme pattern seems to be different in MSCs committed toward differentiation, in which the expression of e-NTPDase or adenosine kinase (the enzyme favoring adenosine phosphorylation and AMP formation) coding genes increases. Since, in general, high levels of extracellular ATP, which accumulates during bone fracture [157], may result dangerous/toxic to cells, the major activity of these enzymes guarantees a tight control of the extracellular nucleotide levels to assure prevalent protective/repairative effects of ATP at low concentrations [158] and to regulate MSC migration *in vitro* or their homing capability *in vivo* [159, 160]. Moreover, also high expression levels of e-5'NT were evidenced in human osteoprogenitor cell lines as well as in human BMSCs and mesenchymal progenitor cells in human trabecular bone [161–163]. The activity of this enzyme contributes to the osteogenic activity induced by transforming growth factor β (TGF β) in human amniotic fluid stem cells [164]. The high expression/function of e-5'NT is coupled to a reduced expression of adenosine deaminase, the enzyme

converting adenosine into inosine, thus assuring appropriate levels and prolonged activity of the extracellular adenosine in MSCs [162].

Finally, outside the cells, purines are catabolized thanks to the activity of membrane (ecto-)enzymes and soluble enzymes similar to those involved in the metabolism and salvage pathway of intracellular purines. Among the cell surface-expressed enzymes, the ecto-nucleotidase 1 (also known as CD39) efficiently hydrolyzes ATP to AMP, whereas the ecto-nucleotidases 2 or 3 and 8 lead to a sustained or transient ADP accumulation, respectively. Then, AMP produced from ATP and ADP degradation is further metabolized by ecto-5'-nucleotidase (also known as CD73) into adenosine. Finally, adenosine is inactivated *via* inosine into hypoxanthine by the tandem action of cell surface-located adenosine deaminase and purine nucleoside phosphorylase or, in part, is transported into the cell to replenish adenine nucleotide pool [132, 134]. Abbreviations: ABCPs, ATP-binding cassette proteins; ADA, adenosine deaminase; AdeSuc, adenylyl-succinate synthase; HGPRT, hypoxanthine/guanine ribosyltransferase; NSkase, nucleoside kinase; NTPDase, Nucleotidase; 5'-NT, 5'-nucleotidase; AMPDA, AMP deaminase; PNP, purine nucleoside phosphorylase; GUADA, guanine deaminase; XO, xanthine oxidase

converting adenosine into inosine, thus assuring appropriate levels and prolonged activity of the extracellular adenosine in MSCs [162].

Expression of purinergic receptors in MSCs

As stated above, purinergic receptors are expressed by almost every cell type and are among the first expressed neurotransmitter receptors during development [136]. They have gained considerable interest since their discovery, and many groups have investigated their potential use as therapeutic targets and/or biomarkers for an array of diseases. P2YR have been deeply explored in the context of thrombosis and heart diseases, whereas P2XR have been studied in inflammatory disorders [159] but also in bone homeostasis [165]. Indeed, a number of studies have demonstrated that, once released, purines bind to the purinoceptors aforementioned, which are present also on MSCs, osteoblasts and osteoclasts, and behave as autocrine/paracrine regulators of bone remodeling [166].

More in detail, the expression of all P1R has been reported in human and rodent MSCs (see Table 1), with a major evidence obtained in BMSCs [162, 172–174]. Apart from a nearly general agreement on the prevalent expression of A2B receptors (A2BR) in MSCs deriving from different sources and committed toward osteogenesis, there may be differences in the expression of the other adenosine receptors. For instance, Hajjawi et al. [176] showed that (i) mRNAs for the A1 receptors (A1R) and A2BR were expressed by all primary rat and mouse osteoblasts, (ii) the expression of A2AR was limited to rat bone marrow and mouse calvarial osteoblasts, and (iii) A3 receptors (A3R) were mainly found in rat bone marrow osteoblasts. In humans, Costa et al. [162] showed a decreased expression of A1R and A2AR along the osteogenic differentiation of MSCs from human aged bone marrow in comparison with that of A2B receptors, being the A3R the less expressed in these cells. More recently, Kotova et al. [197] identified transcripts for A1R, A2AR, and A2BR in human undifferentiated ASCs, whereas no evidence for expression of the A3R was obtained.

As for P2R in general, it has been reported that osteoblast response to nucleotides increases along the osteogenic process, at least in rat MSCs [182]. Looking at the P2X receptors (P2XR), their expression has been found in human MSCs and also in hematopoietic stem cells (HSCs), the extent depending on the stem cell source and the phenotypical commitment toward a specific lineage [196]. In particular, mRNAs for P2X3R, P2X4R, P2X5R, P2X6R, and P2X7R and all P2Y receptors (P2YR) were found in human MSCs, with some peculiarities [196]. For instance, the expression levels of P2Y4R and P2Y12R mRNAs differed between ASCs and ecto-mesenchymal dental follicle cells. Moreover, in studies mainly regarding rat ASCs, P2X6R, and P2Y11R proteins are upregulated upon adipogenic differentiation, while those for P2Y4R and P2Y14R are down-regulated. In contrast, P2X6R mRNA and protein levels decrease in the same ASCs committed toward osteogenesis, so that such a variable expression may be critical for early MSCs commitment to differentiate [198]. In more recent studies, in which human ASCs were again investigated, transcripts for multiple purinoreceptors were found, confirming the presence of P2R previously detected [199], besides most adenosine receptors [197]. In these cells, the effects of specific agonists and antagonists suggested that both P2Y1R and P2Y13R were mainly responsive to ADP, while P2Y4R and P2Y11R served as primary UTP and ATP receptors, respectively. Finally, extracellular NAD^+ stimulated Ca^{2+} signal in ATP-responsive MSCs by interaction with P2Y11.

Influence of P1R on human MSC differentiation

Adenosine modulates different physiological processes in all cells [126]. Its extracellular levels are around 1 μM under

physiological conditions. In case of hypoxia, ischemia, trauma, inflammation, or tumor, this level can increase up to 100-fold [170]. Acting via P1R, adenosine participates in a wide number of processes involved in bone homeostasis such as osteoblastogenesis and osteoclastogenesis [197], as listed below.

A1R

A1R have been investigated mainly in relation to their promotion of osteoclast differentiation. Indeed, A1R selective antagonist, rollofylline, inhibited macrophage colony stimulating factor (M-CSF)/receptor activator of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) ligand (RANKL) or tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6), and TGF- β -activated kinase 1 (TAK1)-induced osteoclastogenesis of mouse bone marrow cells [167]. Moreover, A1R blockade or deletion may increase bone density and prevent ovariectomy-induced bone loss (reviewed in [200]). However, these findings were mostly obtained using rodent MSCs and in vivo animal models.

Focusing on human MSCs, our group [22] and Costa et al. [162] pointed out a proliferative and pro-osteogenic activity of A1R in human MSCs derived from dental pulp or bone marrow, respectively. In particular, while Costa et al. [162] demonstrated a prolonged proliferative effect caused by A1R stimulation along cell osteogenic differentiation, D'Alimonte and colleagues [22] showed that A1R enhanced DPSC proliferation only during the first period along cell differentiation. However, both papers reported that A1R stimulation could also favor the commitment of human MSCs toward osteogenesis. Likely, differences in A1R-mediated effects on cell proliferation could be due to the tissue source from which MSCs derived and the age of patients from whom tissues were obtained (aged vs young people, respectively). It is also to underline that in the case of DPSCs, the authors showed that the enhanced ALP activity, extracellular matrix mineralization, and mRNA expression of Runx2, a key transcription factor in osteogenesis, was coupled to activation of the Wnt signal. Since other studies suggested that the activation of the canonical Wnt pathways could inhibit the differentiation of MSCs [198], they proposed that Wnt signaling may sustain cell differentiation when activated during the early part of this process, inhibiting differentiation when activated later on [23, 201, 202]. More recently, the same authors demonstrated that pro-osteogenic activity of A1R is also evident in human ASCs in which, however, the effect was lower than that observed in DPSCs, since the A1R are expressed to a lesser extent and later during the osteogenic differentiation as compared with DPSCs [70]. Therefore, it seems that the efficacy of A1R stimulation on MSC osteogenic differentiation may depend on (i) the source from which cells derive, which in turn conditions the receptor expression, and (ii) the experimental

Table 1 Principal P1 and P2 purinoreceptor activity in MSCs and bone-related cells

Receptors	Role and function in bone	References	Animal/human Species	Cell origin	Culture conditions	Use of “natural” or “stable” purine compounds
A1	Enhance osteoclastogenesis	[167]	Animal	Bone marrow	Primary cell cultures	Stable
	Promote osteogenic differentiation	[22]	Human	Dental pulp	Primary cell cultures	Stable
		[161]	Human	Bone marrow	Primary cell cultures and immortalized cell lines (HCC1)	Stable
A2A	Reduce inflammatory osteolysis	[162]	Human	Bone marrow	Primary cell cultures	Stable
	Increase osteoblast number while decreasing osteoclast number and activity in inflamed bone	[168]	Animal	Bone marrow	Primary cell cultures	Stable
	Increase the proliferation/development of MSCs	[169]	Animal	Bone marrow	Primary cell cultures	Stable
	Inhibit osteogenic differentiation	[170]	Animal	Bone marrow	Primary cell cultures	Stable
	Enhance osteogenic differentiation	[171]	Human	Bone marrow	Primary cell cultures and immortalized cell lines (MC3T3-E1 cells)	Stable
A2B	Enhance osteogenic differentiation	[172]	Human	Bone marrow	Primary cell cultures	Stable
	Promote osteogenic marker expression	[173, 174]	Animal	Bone marrow	Primary cell cultures	Stable
		[161]	Human	Bone marrow	Primary cell cultures and immortalized cell lines (HCC1)	Stable
	Enhance osteogenic differentiation	[175]	Human	Bone marrow	Primary cell cultures	Natural/stable
	Increase osteogenic differentiation	[162]	Human	Bone marrow	Primary cell cultures	Stable
A3	No effect on osteoblasts or osteoclasts	[176]	Animal	Calvaria bone	Primary cell cultures	Natural/stable
	No activity on osteoblast differentiation	[161]	Human	Bone marrow	Primary cell cultures and immortalized cell lines (HCC1)	Stable
	Decrease osteoclast differentiation improving bone cell survival and proliferation	[177]	Animal	In vivo experiments		Stable
	Decrease bone mineralization and ALP activity	[178]	Animal	Osteoblasts	Primary cell cultures	Stable
	Decrease osteogenic differentiation	[179]	Human	Adipose tissue	Primary cell cultures	Stable
P2X2	Promote bone resorption, increasing osteoclast activity	[180]	Animal	Long bone	Mature osteoclast cultures	Natural
	Decrease osteogenic marker expression in cells from ovariectomized mice	[181]	Animal	Bone marrow	Primary cultures	Stable
P2X3	Decrease bone mineralization	[178]	Animal	Osteoblasts	Primary cell cultures	Stable
	Decrease osteogenic differentiation	[179]	Human	Adipose tissue	Primary cell cultures	Stable
P2X4	Support pore membrane formation together with P2X7R	[181]	Human	Osteosarcoma	Cell lines	Native and stable
P2X5	Stimulate osteoblast proliferation	[182]	Animal	Calvaria bone	Primary cultures	Natural
		[183]	Human	Osteosarcoma	Immortalized cell cultures (MG-63)	Natural/stable
P2X6	Role largely unknown	[10][178]				
P2X7	Physiological stimulation promotes whereas prolonged simulation inhibits osteogenesis	[179]	Human	Adipose tissue	Primary cell cultures	Stable
	Enhance osteoblast apoptosis	[184]	Human	Osteosarcoma and normal bone	Cells lines and primary cell cultures	Natural/stable
	Decrease ALP activity					
	Decrease bone formation	[185]	Animal	Calvaria bone	Primary cell cultures	Natural/stable
	Cause membrane blebbing and bone formation	[186]	Human	Trabecular bone	Primary cell cultures	Stable
		[187]	Human	Bone marrow from post-menopausal women	Primary cell cultures	Stable
	Short (7 days) or prolonged (14 days) cell stimulation increases or decreased ALP activity and osteogenic marker expression	[188]	Animal	Calvaria bone	MC3T3-E1 preosteoblast cell line	Natural/Stable

Table 1 (continued)

Receptors	Role and function in bone	References	Animal/human Species	Cell origin	Culture conditions	Use of “natural” or “stable” purine compounds
P2Y1	Modulate osteoblast response to PTH	[189]	Human	Bone marrow/-osteosarcoma	Primary cell cultures/Sa-OS2 cell lines	Natural
	Favor adipogenic cell differentiation	[175]	Human	Bone marrow	Primary cell cultures	Natural and stable
	Inhibit osteogenesis	[148]	Human	Bone marrow	Primary cell cultures	Native (UTP, UDP) and stable
P2Y2	No activity on osteogenic differentiation	[148]	Human	Bone marrow	Primary cell cultures	Native (UTP, UDP) and stable
	Inhibit osteogenic differentiation	[190]	Animal	Calvaria bone	Primary cultures	Native and stable
	Inhibit bone mineralization and ALP activity favoring adipogenic differentiation via early ERK1/2 activation	[191]	Animal	Bone marrow	Primary cell cultures	Native and stable
P2Y4	Inhibit bone mineralization	[148]	Human	Bone marrow	Primary cell cultures	Native and stable
	Favor adipogenic cell differentiation	[175]	Human	Bone marrow	Primary cell cultures	Stable
		[191]	Animal	Bone marrow	Primary cell cultures	Natural
P2Y6	Increase ALP activity over culture time and intracellular calcium levels	[148]	Human	Bone marrow (young and post-menopausal females)	Primary cell cultures	Native (UTP, UDP) and stable
	Promote early ALP activity and increase of osteogenic marker expression	[188]	Animal	Calvaria bone	Immortalized pre-osteoblast cell lines	Native (UTP) and stable
P2Y11	Largely unknown	[192]	Human	Osteosarcoma	HOS cell lines	-
P2Y12	Inhibit bone mineralization, slow osteoblast proliferation and decrease cell viability.	[193]	Animal	Calvaria bone	Primary cell cultures	Stable (clopidogrel)
	Decrease mineralization in vivo		Animal	Femoral bone and whole body	In vivo model	Stable (clopidogrel)
P2Y13	Promote osteogenic differentiation	[194]	Animal	Bone marrow	Primary cell cultures	Stable
	Increase ALP activity and osteogenic marker expression					
P2Y14	Protect hematopoietic stem cells and may likely enhance osteoclast formation	[195]	KO Animal	Embryos	Total animal or cells derived from them	Radiation
	Likely control MSC commitment decreasing osteogenic differentiation	[196]	Human	Adipose tissue	Primary cell cultures	Stable

models used to obtain results. That A1R may influence also the activity of osteoclasts does not represent an obstacle since the receptor activity should be considered in a more general context in which, in example, the effects exerted by the activity of these receptors on osteoblast and osteoclast functions may be regarded as an attempt by the same ligand (adenosine) to balance the tasks of the two cell types in bone remodeling.

A2AR and A2BR

A2AR activation displays different effects as compared with those consequent to A1R stimulation, A2AR being able to inhibit mouse osteoclast differentiation and to enhance bone density, likely through disruption of M-CSF/RANKL ratio, decrease of interleukin 1 β (IL-1 β) and TNF-alpha levels, and inhibition of cAMP/protein kinase A (PKA)/ERK1/2 signaling pathway [168]. A2AR may also play an active role in mouse BMSC development [155] and their stimulation

reduces inflammatory osteolysis, increasing osteoblast numbers in inflamed bone [169]. As well, in a bone defect condition, A2AR help restore bone homeostasis by increasing osteoblasts while decreasing osteoclast number and activity [203]. In contrast, He and colleagues observed that activation or downregulation of the A2AR did not affect human osteoblast differentiation and mineralization [172], whereas Costa et al. [162] demonstrated an inhibitory A2AR effect on the osteogenic differentiation of BMSCs from osteoporotic female patients. Recently, Borhani et al. [171] showed in a murine osteoblast precursor cell line (MC3T3-E1) and in primary mouse osteoblasts that A2AR activation promotes Akt signal, enhancing β -catenin nuclear translocation that may likely increase cell osteogenic differentiation. Once again, the results about A2AR function and bone plasticity have mostly been obtained using animal MSCs/models. Thus, the role of this receptor in osteoblast biology remains ambiguous and not clarified and, overall, further evidence is needed in humans.

Differently from A2AR, several research groups demonstrated that adenosine enhances osteoblast differentiation mainly by interacting with the A2BR [162, 172–174]. Even though A2BR show a low affinity for the endogenous ligand adenosine, the observed increase in extracellular adenosine concentration as well as sensitization/upregulation of A2BR expression under conditions of hypoxia and inflammation suggests the A2BR could be an interesting therapeutic target in different pathological states, including bone disease. In 2006, Evans et al. demonstrated that A2BR were the functionally dominant P1 receptors in human osteoprogenitor cells and also in a human immortalized cell line [161], which were able to secrete adenosine *in vitro*. A2BR activation enhanced cell osteogenic differentiation by upregulating the expression of typical osteogenic markers like Runx2. Accordingly, BMSCs from A2BR knockout mice showed a reduced osteogenic potential and the same knockout animals exhibited a lower bone density and a delayed fracture repair [204]. Stimulation of A2BR also attenuated bone loss in ovariectomized mice, highlighting the potential of A2BR as a target to treat osteoporosis consequent to estrogen deficiency [205]. Thus, A2BR deserve a particular attention, since they result to be the most effective among adenosine-responsive receptors in increasing the osteogenic differentiation of MSCs, both in those derived from aging females [162] or rodents [173, 174]. However, all findings are related to MSCs from bone marrow; thus, information on other MSC types are lacking. A2BR stimulation in BMSCs may also mediate MSC differentiation by modulating interleukin 6 (IL-6) levels. In the first stage of differentiation, A2BR reduce IL-6, which favors MSC commitment to osteoblasts, but, in the last phase, these receptors cause release of IL-6, which then promotes survival of differentiated MSCs. For this reason, IL-6 restoration by A2BR agonists could promote osteoblast viability and may be used in regeneration and tissue repair. In contrast to the general consensus on the proosteogenic role played by A2BR, Hajjawi and colleagues showed that rodent osteoblast numbers and differentiation were unaffected by high/not physiological concentrations of adenosine or an A2BR agonist (BAY606583). The authors argued that differences in culture methods may contribute to these findings [176].

A3R

Among the adenosine receptors, the role of A3R in osteoblast differentiation remains widely unknown. Our group has demonstrated the expression of A3R in human DPSCs [22]. Evans et al. also showed that A3R are expressed by human osteoblast precursors and osteoblasts [161], even though the use of the A3R agonist, N⁶-(3-iodo benzyl)adenosine-5'-N-methyluronamide (IB-MECA) did not affect osteoblast cell differentiation measured in terms of ALP activity. In contrast, A3AR activation has been correlated to decreased osteoclast

differentiation due to phosphoinositide 3-kinase (PI3K), NFkB, and RANKL downregulation [177]. In addition, IB-MECA modulated the expression of proteins which control survival and apoptosis resulting in the improvement of the inflammatory process and the preservation of bone mass in adjuvant induced arthritis rats. Since these receptors play a major role in inflammatory diseases, it is likely that the experimental conditions to study their involvement in MSC differentiation still need to be better identified.

In conclusion, adenosine receptor activation is involved in bone remodeling/repair, being A2BR and likely A1R able to promote and to favor, respectively, MSC osteogenic differentiation. Therefore, they represent a potential tool that should be, however, better explored for bone regenerative therapies.

Influence of P2R on human MSC differentiation

As mentioned above, nucleotide receptors play important roles in the functions of both osteoblasts and osteoclasts. *In vitro* cultures have been used to elicit the activity of endogenous ATP in MSCs and HSCs, respectively [165]. Thus, it was shown that low-intensity pulsed ultrasound stimulation treatment, which induces ATP release from osteoblasts, also caused a subsequent activation of P2R-mediated proliferation [150]. As well, bisphosphonates, used for the treatment of osteoporosis, promote nonlytic release of ATP and also the activation of its receptors, namely P2Y1R and P2Y2R, which in turn lead to activation of ERKs [206]. ATP release may be induced also by P2Y2R stimulation by uridine-based nucleotides, even though this has been shown only in osteoclasts [207]. Additionally, an increasing number of findings are showing the ability of these receptors to modulate the osteogenic differentiation of MSCs, thus providing new avenues by which they can be manipulated for tissue regenerative strategies.

Concerning metabotropic P2YR, many authors have demonstrated different effects in bone remodeling, as follows:

- the P2Y1R were shown to modulate human osteoblast responses to systemic factors, such as parathyroid hormone (PTH) [189]; however, other reports indicated that the stimulation of these receptors led to inhibition of osteogenesis in human ASCs and BMSCs [148, 187, 196], even favoring the adipogenic differentiation of the latter [175];
- regarding P2Y2R and P2Y4R, Hoebertz et al. found that ATP and UTP at concentrations $\geq 1\mu\text{M}$ are potent inhibitors of mineralization in cultured primary rat calvarial osteoblasts [190]. Recently, Du et al. [208] reported that overexpressing or silencing of P2Y2R impaired or promoted osteogenic differentiation of mouse MSCs, respectively. Since the expression of P2Y2R was much higher in osteoblasts from ovariectomized mice in comparison with

control, the authors suggest that their suppression might be an effective strategy to attenuate the estrogen deficiency-induced osteoporosis. Data in human BMSCs showed an inhibitory activity of P2Y2R on cell osteogenic differentiation [148], and are in agreement with findings obtained in rat BMSCs [191], being these receptors and not P2Y4R able to favor the adipogenic differentiation of both cells;

- the expression of the P2Y6R is fairly constant throughout maturation of human cultured BMSCs from postmenopausal women, and their stimulation promotes ALP activity and increase in intracellular $[Ca^{2+}]$ [148]; similar findings have been obtained from immortalized cell lines of mouse calvaria bone [188];
- the expression of the P2Y11R was detected by RT-PCR in a human osteosarcoma cell line (HOS cells), but their function remains elusive [188];
- the ADP-sensitive P2Y12R are usually associated to bone nodule formation [192]. This association also came from the clinical use of a selective P2Y12R antagonist (clopidogrel) that was able to inhibit in vitro mineralization in a concentration-dependent manner, slowing osteoblast proliferation and cell viability, together with a reduction in ALP activity and collagen formation [193];
- the ADP-sensitive P2Y13R might be involved in bone remodeling. In fact, ADP activation of P2Y13R+/+ (but not P2Y13R-/-) adherent BMSCs from mice significantly increases the number of alkaline phosphatase-colony-forming units (CFU-ALP) as well as the expression of some osteoblast markers (Osx, ALP and type I collagen) [194];
- the role of P2Y14R in bone remodeling also remains still elusive. Some authors suggested that these receptors preserve hematopoietic stem/progenitor cell function [195], whereas others indicated that, acting together with P2X6R and P2Y4R, they may control human ASC commitment toward both adipogenic and osteogenic differentiation. In bone cells, the P2Y14R expression decreases along the passage from undifferentiated to osteoblast-differentiated cells, suggesting an important role of them at early differentiation stages [reviewed by [196].

Furthermore, there are many reports elucidating the role played by ionotropic P2XR on MSC osteogenic differentiation. In particular:

- Orriss et al. [178] demonstrated that, in osteoblasts from neonatal Sprague–Dawley rats, P2X1R and P2X3R are involved in the prevention of bone nodule mineralization in vitro, since the P2X1R and P2X3R agonists, α, β -meATP, and β, γ -meATP, respectively, inhibited bone mineralization. More recently, Carluccio et al. [179] confirmed the inhibitory activity of both receptors. Indeed,

they demonstrated in human ASCs committed toward osteogenesis the expression of both receptor types, even though to a lower extent as compared with P2X7R (see below), also present in the same cells; moreover, cell exposure to 2'(3')-O-(4-benzoylbenzoyl) adenosine 5'-triphosphate (Bz-ATP) decreased ASC osteogenic differentiation interacting with P2X1/3R.

- On the other hand, the P2X2R seem to be more important in rodent osteoclasts than in osteoblasts, as they promote bone resorption [180]. More recently, Kanaya et al. [181] also showed a decrease in osteogenic marker expression using BMSCs from ovariectomized mice.
- The P2X4R are expressed in osteoblasts, however derived from human osteosarcoma cell lines, and seem to be involved in membrane pore formation. Indeed, in these cells, the non-selective P2X7R agonist, BzATP, induced the incorporation of a fluorescent dye (a powerful indicator of pore formation). However, P2X7R antagonists were ineffective to completely block pore formation, thus suggesting that P2X4R may be also involved in this mechanism [209].
- Nakamura et al. showed that ATP, acting on P2X5R, among others, can stimulate osteoblast-like (MG-63) cell proliferation, via a mechanism that is dependent on MAPK pathway [183]. Accordingly, Orriss et al. [182] reported a similar observation using MSCs from rat calvaria bone.
- The expression of the P2X6R has also been detected in primary cultures of rat and human (SaM-1) osteoblast-like cells, but their role in bone formation is largely unknown [210].
- Finally, the role of the P2X7R in osteoblast function appears important but controversial. Early studies suggested that P2X7R activation caused enhanced human osteoblast apoptosis [184]. In contrast, more recent studies indicate that P2X7R stimulation leads to increased membrane blebbing of mouse MSCs and bone formation [211–213]. These roles are supported by P2X7R knockout murine models, where decreased bone mass has been reported as compared with wild-type animals [214]. Moreover, a great number of functional single nucleotide polymorphisms (SNPs) [215], leading to either gain or loss of function of the P2X7R protein, has been identified. Many of these SNPs have been associated with bone alterations in humans. Indeed, gain-of-function polymorphisms of P2X7R have been coupled to increased bone mass [216], whereas loss-of-function polymorphisms of this receptor have been associated with increased fracture risk, reduced bone mineral density and osteoporosis in postmenopausal women [185, 217, 218], and stress fracture injury in military personnel and elite athletes [219].

Summarizing, as for P1R, also for P2R, the studies highlighted a number of interactions between nucleotide signaling and MSCs within the context of bone development and homeostasis. However, the results were once again obtained in cells *in vitro*, and mostly in cells derived from rodent tissues. Thereby, the translation of these results to *in vivo* models is compelling.

Finally, it is to recall that, since pathological processes underlying osteoporosis and other bone defects may be due to an imbalance between osteogenesis and adipogenesis, A2AR, within the P1R family, enhance the MSC adipogenic differentiation, whereas A2BR inhibit this process and A1R stimulation mainly controls adipocyte lipogenesis [173, 220]. P2R may also direct MSCs toward an adipogenic differentiation. P2Y11R are important players in this process, and also, P2Y1R and P2Y4R may stimulate MSC adipogenesis [175], whereas most P2R substantially favor osteogenesis and inhibit the adipogenic differentiation (see [11]).

In Table 1 the main findings are summarized concerning P1R and P2R functions in MSCs submitted to differentiation, with a particular attention toward the osteogenic one.

In conclusion, the most promising receptors favoring MSC osteogenic differentiation seem to be A2BR, P2Y6R, and mainly P2X7R. While data related to the first two receptor types are still few and essentially limited to *in vitro* studies, in the next pages we reported more detailed experimental observations on P2X7R and osteogenesis, since there is a great interest in the role of these receptors in modulating bone formation and resorption processes and studies have been conducted in preclinical animal models and in humans. However, due to the great effervescence in this field, results so far obtained are numerous but often conflicting. Thus, in the next chapter, we tried to arrange a unifying vision of the activity of these receptors in bone homeostasis/repair, based also on our personal experience.

Focus on P2X7R

P2X7R are very particular ATP-gated, non-selective cation channels belonging to the family of ionotropic P2XR. *P2RX7* gene encodes a 595-aa protein (the P2X7R subunit or monomer) that assembles into a trimeric complex to form the functional P2X7R [221]. Interestingly, human P2X7R exhibit a number of non-synonymous SNPs, which results in a change in amino acid sequence and expression of different human *P2X7R* variants, further increasing the structural diversity of these receptors [222]. In humans, seven naturally occurring splice variants were initially reported and their monomeric subunits were termed as P2X7B up to P2X7H [223], with P2X7A referring to the original full-length 595 amino acid protein [224]. Among them, there are truncated variants with an alternate short C terminus (P2X7B, C, E, and G),

variants that lack exons responsible for coding parts of the extracellular domain (P2X7C–F) and variants with additional exons, thought to result in P2X7 protein lacking the first transmembrane domain (P2X7G and H) [225]. Two additional naturally occurring splice variants have been identified in humans, termed P2X7I and P2X7J [226]. These ten variants of human P2X7R subunits differ in distribution, functional characteristics, and predicted physiologic roles [227]. Of these, P2X7B is the best studied and, when assembled into a homomeric P2X7BR, it is able to form functional channels but not the large pores that are often associated with inflammation and cell death [228]. Interestingly, P2X7R polymorphisms are associated with low mineral density of lumbar spines and increased bone loss in postmenopausal women [229].

Several pharmacological differences exist between human and rodent P2X7R, including varying EC_{50} values for the agonists ATP and BzATP (10- to 25-fold higher for humans, in whom P2X7R are activated by millimolar concentrations of extracellular ATP) and sensitivity to other compounds. The agonist profile for human P2X7R is BzATP >> ATP > 2MeSATP > ATP γ S >> ADP [230]. Upon ligand binding, P2X7R facilitates the influx of Ca²⁺ and Na⁺ and the efflux of K⁺. However, P2X7R overstimulation by elevated ATP concentrations, like those found during inflammatory events, triggers the opening of a non-selective plasma membrane pore, known as a “macropore,” which allows fluxes of large hydrophilic molecules. This can lead to diverse responses including activation of MAPKs and gene transcription, activation of the inflammasome, as well as the subsequent processing and release of the important inflammatory cytokine IL-1 β , or sometimes activation of caspases and apoptosis depending on the given cell type and the experimental conditions [231, 232].

Role of P2X7Rs in bone cell formation and function

Studies so far performed pointed out that the expression and function of P2X7R vary depending on the stage and on the species of bone cells and that P2X7R are able to either promote or inhibit bone mineralization in osteoblasts [233].

Specifically, ATP stimulation of P2X7R can lead to the induction and/or activation of the activating protein-1 (AP-1) transcription factors, JunB, c-Fos, FosB, and Δ FosB [234]. The AP-1 transcription factors, in addition to Runx2, Distal-less homeobox 5 (Dlx5), Msx2, and Osx, play critical roles in osteoclast and osteoblast formation [235]. These gene regulatory proteins are known to be modulated by several components of the Wnt signaling pathway involved in bone formation. Wnt signaling occurs via both canonical (Wnt3a/ β -catenin) and non-canonical molecular pathways. It has been reported that the non-selective P2X7R agonist, BzATP, inhibits β -catenin nuclear translocation, significantly decreases ALP activity in primary rat calvarial osteoblasts [185], and reduces

nodule formation in primary human trabecular osteoblasts [186]. Moreover, P2X7R activation inhibits human mandibular-derived osteoblast differentiation by down-regulating the canonical Wnt signaling pathway [236].

In contrast, P2X7R were also found to have a stimulating, rather than an inhibiting effect on *in vitro* mineralization [179, 187, 188, 211]. Promotion of cell osteogenic differentiation both in animal and human tissue-derived MSCs by P2X7R was mediated by activation of molecular pathways leading to the production of lipid mediators such as lysophosphatidic acid and prostaglandin E₂ [211] as well as to cell membrane blebbing (zeiosis) [187, 211] in a Rho kinase-dependent manner. In BMSCs obtained from human post-menopausal female, this mechanism has also been linked to activation of phospholipase C and PKC [187].

The differences emerged from the examined papers as for the response of MSC osteogenic differentiation to P2X7R activation could reside on issues such as:

- different experimental models, in terms of animal species and tissue sources from which MSCs were obtained. As above reported, cells under investigation were obtained from animals or humans. From those studies, it could be inferred that cells deriving from rodent [211] or human bone marrow (healthy young or aged diseased females) [187] responded to prolonged P2X7R stimulation with an increased osteogenic differentiation, whereas those from rodent [188] or human [179, 236] tissues different from bone marrow underwent a decrease in their osteogenic differentiation potential. These findings could be likely related to a diverse expression and/or function of P2X7R in those cells [179, 187] or, also, to a different inactivation of the P2X7R agonist, BzATP, by ecto-NTDPases [10];
- different concentrations of agonists assayed in the experiments. In this concern, the use of ATP or BzATP concentrations [179, 186, 236], unable to selectively stimulate only P2X7R, could elicit the activity of other P2R with negative influence on osteogenic differentiation.

Anyway, today, it seems that the pro-osteogenic activity mediated by P2X7R is better characterized. Thus, in patients with nonunion bone fractures, shockwave therapy enhanced bone healing via ATP release from hMSCs and the subsequent activation of P2X7R, responsible for hMSCs osteogenic differentiation as well as osteoblast proliferation and mineralization. Interestingly, pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS), a pan P2X antagonist, the human P2X7 antagonist (4-[(2*S*)-2-[(5-isoquinoliny)sulfonyl)methylamino]-3-oxo-3-(4-phenyl-1-piperazinyl)propyl] phenyl isoquinolinesulfonic acid ester (KN-62) and a P2X7R-specific siRNA strongly reduced or completely abolished these effects. Shockwave-induced effects were also prevented by apyrase, supporting a role

for endogenously released ATP [237]. In addition, the truncated P2X7R isoform, P2X7BR, when co-expressed with the full variant P2X7AR, demonstrates a significant enhancement of mineralization in human osteosarcoma cell line [238], thus consolidating a positive role for the fully functional P2X7R in maintenance of bone strength.

The molecular mechanism underlying this beneficial effect would include calcium mobilization [239] and activation of MAPK/c-Jun NH₂-terminal kinase (JNK) signaling cascade [240]. Indeed, several studies support a role for the P2X7R and MAPK signaling in osteogenic differentiation of human MSCs [241]. Cell treatment with KN-62, which, besides P2X7R also blocks Ca²⁺/calmodulin-dependent kinase type II, or with the non-selective P2R antagonist, PPADS, suppressed p38/MAPK dependent osteogenic differentiation of MSCs in response to shockwaves and ATP treatment [237].

The involvement of P2X7R and endogenous ATP was also shown in murine MC3T3-E1 osteoblastic cells subjected to tension force, in which the P2X7R antagonist 3-[[5-(2,3-dichlorophenyl)-1*H*-tetrazol-1-yl]methyl]pyridine hydrochloride (A438079) blocked the expression of Runx2 [242]. However, studies by Ciciarello and colleagues [175] demonstrated that Runx2 mRNA levels are lower in differentiated human BMSCs treated with inhibitors against the ecto-NTPDase/CD39 and the ecto-5'NT/CD73. This finding indicates that Runx2 expression may be regulated by the ATP breakdown product adenosine instead of ATP. Accordingly, Carluccio et al. [179] recently reported that the P2X7R antagonist A438079, when administered alone to human ASCs (derived from healthy young females, BMI < 25) first decreased, whereas then increased their osteogenic differentiation. This latter effect was similar to that shown in the same cells by apyrase, an enzyme accelerating the conversion of nucleotides into adenosine. In an attempt to find a unifying explanation, it is conceivable that the interaction of endogenous ATP with P2X7R, the expression of which significantly increased during ASC differentiation, underlays early commitment of these cells toward osteogenesis, whereas adenosine, derived from ATP metabolism, is crucial in the second phase of this process. The pro-osteogenic activity of adenosine (delayed in ASCs) might be reasonably related to the nucleoside interaction with A1R and mainly with A2BR, as previously reported [70, 173, 174]. If confirmed in other MSCs, this would mean that ATP and adenosine may cooperate in concerted manner over time to assure the correct/complete differentiation of engaged MSCs into osteoblasts (see Fig. 3).

It is also to underline that during ASC osteogenic differentiation, the expression of P2X7R A and B splice variants significantly increased. This finding can represent a turning point in the investigation on the role played by P2X7R in osteogenesis, since the co-expression of A and B subunits of the P2X7R may favor the assembly of a heterotrimeric

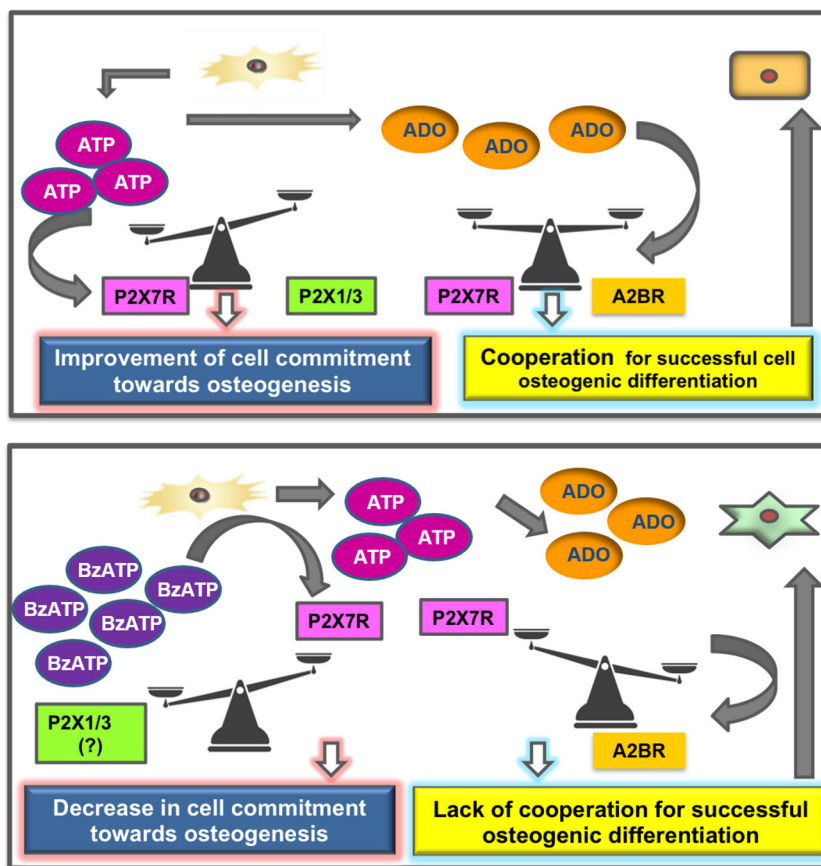


Fig. 3 Scheme for a possible cooperation between endogenous ATP and adenosine in modulating the osteogenic differentiation of ASCs. **a** Endogenous ATP, released from normal and even more from injured tissue, may interact with P2X7R; the expression of which is significantly increased during ASC osteogenic differentiation, thus supporting this process. The activity of adenosine, derived from ATP metabolism, is more evident in a second time, in which the nucleoside should interact with its receptors (likely of A2BR subtype [173, 174]). These data, if confirmed in MSCs from other human sources, would suggest a temporal cooperation between endogenous ATP and

adenosine to assure the correct/complete differentiation of engaged MSCs into osteoblasts. **b** In contrast, BzATP, a non-selective P2X7R agonist, when administered along cell differentiation, inhibits ASC commitment toward osteogenesis. This inhibitory effect could be either due to its interaction with other P2XR (i.e., P2X1R and P2X3R, mainly expressed in ASCs during the first phase of their osteogenic differentiation) or to a decreased P2X7R expression [179]. In this condition, endogenous adenosine is no longer able to increase the ASCs osteogenic differentiation

receptor with a greater sensitivity toward ligands, which may be able to better support the energy supply to cells [232] and likely their function. Conversely, ASC exposure to a prolonged pharmacological stimulation with the non-selective agonist BzATP (added to cells under osteogenic differentiation at each medium change, that is, every 3 days, along a period of 28 days) decreased both P2X7R expression and osteogenic differentiation. Such a P2X7R downregulation, already observed by Rodrigues-Ribeiro et al. [188], should be not considered in contrast to the often reported non-desensitization of P2X7R, even to high agonist concentrations, since these data are generally referred to cell exposure to P2X7R agonist for a brief period (minutes) and in relation to ion channel/pore opening [243]. In our case, the drug addition (BzATP 100 μ M) to ASCs was repeated several times along a period of 28 days. Thus, the decreased P2X7R expression can be regarded as a defensive tentative by cells against

such a repeated, potentially toxic, pharmacological treatment. In agreement with the overall data by Carluccio et al. [179], a number of studies today support the idea that while the basal/transient activation of P2X7R is osteogenic, sustained stimulation could inhibit their expression/function and, thereby, their activity on bone-forming cells [244], favoring negative effects by other P2R such as P2X1/3R, as previously mentioned.

Conclusions

Summarizing data herein reported have highlighted that purines and their receptors can affect MSC proliferation/differentiation potential. But, in our opinion, the scenario in which MSCs and purines move is complex, so that many factors should be taken into consideration.

First of all, it is still difficult to identify the best way to pursue for a successful use of MSCs in bone repair [245]. In our opinion, a general agreement is needed within the scientific community both to standardize the techniques and methods to obtain “safe” MSCs to use for osteogenic purposes and to identify preclinical models suitable to obtain reliable results that can be translated to humans for regenerative medicine. This aspect is crucial and without this agreement, research will inevitably proceed in a sparse way and it will be difficult to arrive to a clear understanding of its usefulness [246]. Obviously, data obtained from the use of immortalized cell lines are of interest, but only as producing pivotal findings, which should be confirmed in normal (possibly human) MSCs.

As for purines, findings so far obtained in relation to their modulation of MSC osteogenic differentiation have been regarded as promising, but, also from data herein reported, it is evident the need to implement studies *in vitro* on human MSCs from different tissue sources and mainly *in vivo*, to achieve a more uniform body of evidence, especially for those purine receptors mostly involved in promoting osteogenesis and bone repair processes.

As well, in future studies, it should also be taken into consideration the tight functional interplay existing among intra- and extracellular levels of purines, their receptors, degrading inside- and ecto-enzymes and bidirectional transport systems, all together forming the so purine called “purinome” [10, 138]. This network should be better elucidated in different MSCs so far evaluated during the differentiation processes, to understand whether dysfunction in this system may alter MSC contribution to osteogenesis and, consequently, to adopt therapeutic strategies to correct eventual derangements. The purinome should be also investigated in MSCs either moving from endogenous bone niches or exogenously implanted in the injured/inflamed tissue, in which the extracellular purine amount is increased by different mechanisms (see previous paragraphs). Clearly, in these conditions, the machinery to degrade purines is activated, but its function should be controlled to assure adequate quantities of adenine nucleotides and nucleosides able to promote pro-osteogenic and anti-inflammatory stimuli [200].

Finally, recent preclinical and clinical studies on stem cell-based therapy are pointing out that the success of this application in regenerative medicine is mostly due to factors secreted from exogenous MSCs rather than to differentiation of implanted stem cells in targeted tissues [140, 247, 248]. These substances represent the so called “secretome,” which is defined as the set of factors/molecules secreted from cells to the extracellular space, including, among others, soluble proteins, free nucleic acids, lipids, and extracellular vesicles, which are further classified into distinct groups (exosomes and microvesicles) and are attracting a growing interest [249]. The secretome of individual cells and tissues is specific

and changes in response to fluctuations in physiological states or pathological conditions [250].

As a consequence, the investigations so far performed on the purine ability to affect MSC proliferation/osteogenic differentiation potential should be regarded more as a tentative to explain the influence of purines on endogenous MSCs in bone physiology/diseases rather than a real attempt to improve the use of exogenous MSCs in bone regenerative medicine. Thus, it is reasonable to foresee that in the next future the research on purines should be addressed to understand if purines are important compounds within the secretome and how purines, by interacting with their receptors, may affect substances released from MSCs able to favor bone healing/repair. In other words, it has become imperative to evaluate purine involvement in and interactions with MSC secretome, the knowledge of which is at the very beginning [251, 252].

The road ahead is still long but, hopefully, studies in this direction should be more useful to discover the real potentialities of using both MSCs and purines.

Acknowledgments This work was supported by funds for research to RC and PDI from the University of Chieti-Pescara.

Authors' contributions MC drafted the manuscript together with RC. SZ, MZ, and PG collected papers for the different sections of the manuscript. PDI and FC contributed to analyze data from collected papers. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest statement The authors declare no existing conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Abbreviations A438079, 3-[[5-(2,3-dichlorophenyl)-1H-tetrazol-1-yl]methyl]pyridine hydrochloride; *ABMSCs*, alveolar bone-derived MSCs; *AP-1*, activating protein-1; *ALP*, alkaline phosphatase; *AIR*, A2AR, A2BR, A3R, adenosine A1, A2A, A2B, A3 receptors; *ASCs*, adipose tissue-derived stem cells; *ATP*, *GTP*, *UTP*, *ADP*, adenosine, guanosine, uridine tri- or di-phosphate; *BDNF*, brain-derived growth factor; *BMSCs*, bone marrow-derived stem cells; *BzATP*, 2'(3')-O-(4-benzoylbenzoyl)adenosine 5'-triphosphate triethylammonium; *cAMP*, adenosine 3',5' cyclic monophosphate; *cGMP*, guanosine 3',5' cyclic monophosphate; *CM*, conditioned medium; *DFPCs*, dental follicle progenitor cells; *DPSCs*, dental pulp stem cells; *DSCs*, dental stem cells; *ECM*, extracellular matrix; *ECs*, endothelial cells; *e-5'NT*, ecto-5' nucleotidase; *ERK1/2*, extracellular signal-regulated kinase 1 and 2; *G-CSF*, granulocyte colony-stimulating factor; *GFs*, growth factors; *GMSCs*, gingival MSCs; *HGF*, hepatocyte growth factor; *HSCs*, hematopoietic stem cells; *JNK*, c-Jun NH₂-terminal kinase; *KN-62*, [(2S)-2-[(5-isoquinolinylsulfonyl)methylamino]-3-oxo-3-(4-phenyl-1-piperazinyl)propyl] phenyl isoquinolinesulfonic acid ester; *IB-MECA*, N⁶-(3-iodobenzyl)adenosine-5'-N-methyluronamide; *IL-1*, -6, -8, interleukin 1, 6 and 8; *iPS*, pluripotent stem cells; *MAPK*, mitogen activated protein kinase; *M-CSF*, macrophage colony stimulating factor; *MOBL*, mesenchymal osteoblasts; *MSCs*, mesenchymal stem cells; *Msx1/2*, mouse segment homeobox 1/2; *NF-κB*, nuclear factor kappa-light-chain-enhancer of

activated B cells; *NTPDase*, nucleoside triphosphate diphosphohydrolase; *Osx*, osterix; *PI3K*, phosphoinositide 3-kinase; *PKA*, protein kinase A; *PDLSCs*, periodontal ligament stem cells; *PIR-P2R-P2XR-P2YR*, P1-P2-P2X-P2Y receptors; *PPADS*, pyridoxal phosphate-6-azophenyl-2',4'-disulfonic acid; *PTH*, parathyroid hormone; *P2X7R*, P2X7 receptors; *RUNX2*, runt-related transcription factor 2; *SAT*, subcutaneous adipose tissue; *SCAP*, stem cells from apical papilla; *SCI*, spinal cord injury; *SHED*, exfoliated deciduous teeth; *SOBL*, surface osteoblasts; *SVF*, stromal vascular fraction; *TCF/LEF*, T-cell factor/lymphoid enhancer factor; *TNF*, tumor necrosis factor; *TGF- β* , transforming growth factor beta; *TGPCs*, tooth germ progenitor cells; *VAT*, visceral adipose tissue

References

- Verrier S, Alini M, Alsberg E et al (2016) Tissue engineering and regenerative approaches to improving the healing of large bone defects. *Eur Cell Mater* 32:87–110
- Ganguly P, El-Jawhari JJ, Giannoudis PV et al (2017) Age-related changes in bone marrow mesenchymal stromal cells: a potential impact on osteoporosis and osteoarthritis development. *Cell Transplant* 26(9):1520–1529. <https://doi.org/10.1177/0963689717721201>
- Lechner J, Aschoff J, Rudi T (2018) The vitamin D receptor and the etiology of RANTES/CCL-expressive fatty-degenerative osteolysis of the jawbone: an interface between osteoimmunology and bone metabolism. *Int J Gen Med* 11:155–166. <https://doi.org/10.2147/IJGMS152873>
- Amini AR, Laurencin CT, Nukavarapu SP (2012) Bone tissue engineering: recent advances and challenges. *Crit Rev Biomed Eng* 40:363–408
- Oryan A, Kamali A, Moshiri A, Baghaban Eslaminejad M (2017) Oryan A et al. Role of mesenchymal stem cells in bone regenerative medicine: what is the evidence? *Cells Tissues Organs* 204(2):59–83. <https://doi.org/10.1159/000469704>
- Caplan AI (2017) Mesenchymal stem cells: time to change the name! *Stem Cells Transl Med* 6:1445–1451. <https://doi.org/10.1002/sctm17-0051>
- Cavaliere F, Donno C, D'Ambrosi N (2015) Purinergic signaling: a common pathway for neural and mesenchymal stem cell maintenance and differentiation. *Front Cell Neurosci* 9:211. <https://doi.org/10.3389/fncel.2015.00211>
- Jiang LH, Hao Y, Mousawi F, Peng H, Yang X (2017) Expression of P2 purinergic receptors in mesenchymal stem cells and their roles in extracellular nucleotide regulation of cell functions. *J Cell Physiol* 232:287–297. <https://doi.org/10.1002/jcp.25484>
- Kaebisch C, Schipper D, Babczyk P, Tobiasch E (2015) The role of purinergic receptors in stem cell differentiation. *Comput Struct Biotechnol J* 13:75. <https://doi.org/10.1016/j.csbj.2014.11.003>
- Noronha-Matos JB, Correia-De-Sá P (2016) Mesenchymal stem cells ageing: targeting the “purinome” to promote osteogenic differentiation and bone repair. *J Cell Physiol* 231(9):1852–1861. <https://doi.org/10.1002/jcp.25303>
- Roszek K, Wujak M (2019) How to influence the mesenchymal stem cells fate? Emerging role of ectoenzymes metabolizing nucleotides. *J Cell Physiol* 234:320–334
- Schönherr E, Haussler HJ (2000) Extracellular matrix and cytokines: a functional unit. *Dev Immunol* 7(2–4):89–101
- Clarke B (2008) Normal bone anatomy and physiology. *Clin J Am Soc Nephrol CJASN* 3(Suppl 3):S131–S139
- Schaffler MB, Cheung WY, Majeska R, Kennedy O (2014) Osteocytes: master orchestrators of bone. *Calcif Tissue Int* 94(1):5–24. <https://doi.org/10.1007/s00223-013-9790-y>
- Manolagas SC, Parfitt AM (2010) What old means to bone. *Trends Endocrinol Metab* 21(6):369–374. <https://doi.org/10.1016/j.tem.2010.10.010>
- Bonewald LF (2011) The amazing osteocyte. *J Bone Miner Res* 26(2):229–238. <https://doi.org/10.1002/jbmr.320>
- Infante A, Rodríguez CI (2018) Osteogenesis and aging: lessons from mesenchymal stem cells. *Stem Cell Res Ther* 9:244. <https://doi.org/10.1186/s13287-018-0995-x>
- Shapiro F (2008) Bone development and its relation to fracture repair. The role of mesenchymal osteoblasts and surface osteoblasts. *Eur Cell Mater* 15:53–76
- Birmingham E, Niebur GL, McHugh PE et al (2012) Osteogenic differentiation of mesenchymal stem cells is regulated by osteocyte and osteoblast cells in a simplified bone niche. *Eur Cell Mater* 23:13–27
- Rao TP, Kühl M (2010) An updated overview on Wnt signaling pathways: a prelude for more. *Circ Res* 106(12):1798–1806
- Krishnan V, Bryant HU, Macdougald OA (2006) Regulation of bone mass by Wnt signaling. *J Clin Invest* 116:1202–1209
- D'Alimonte I, Nargi E, Lannutti A et al (2013) Adenosine A1 receptor stimulation enhances osteogenic differentiation of human dental pulp-derived mesenchymal stem cells via WNT signaling. *Stem Cell Res* 11(1):611–624. <https://doi.org/10.1016/j.scr.2013.04.002>
- D'Alimonte I, Lannutti A, Pipino C et al (2013) Wnt signaling behaves as a “master regulator” in the osteogenic and adipogenic commitment of human amniotic fluid mesenchymal stem cells. *Stem Cell Rev* 9(5):642–654. <https://doi.org/10.1007/s12015-013-9436-5>
- Siddappa R, Doorn J, Liu J et al (2010) Timing, rather than the concentration of cyclic AMP, correlates to osteogenic differentiation of human mesenchymal stem cells. *J Tissue Eng Regen Med* 4(5):356–365
- Siddappa R, Martens A, Doorn J et al (2008) cAMP/PKA pathway activation in human mesenchymal stem cells in vitro results in robust bone formation in vivo. *Proc Natl Acad Sci* 105(20):7281–7286
- Wang FS, Wang CJ, Sheen-Chen SM et al (2002) Superoxide mediates shock wave induction of ERK-dependent osteogenic transcription factor (Cbfa1) and mesenchymal cell differentiation toward osteoprogenitors. *J Biol Chem* 277:10931–10937
- Kim SH, Choi YR, Park MS et al (2007) ERK1/2 activation in enhanced osteogenesis of human mesenchymal stem cells in poly(lactic-glycolic acid) by cyclic hydrostatic pressure. *J Biomed Mater Res A* 80:826–836
- Jensen ED, Gopalakrishnan R, Westendorf JJ (2010) Regulation of gene expression in osteoblasts. *Biofactors* 36:25–32. <https://doi.org/10.1002/biof72>
- Zhou X, Zhang Z, Feng JQ et al (2010) Multiple functions of Osterix are required for bone growth and homeostasis in postnatal mice. *Proc Natl Acad Sci USA* 107:12919–12924. <https://doi.org/10.1073/pnas.0912855107>
- Royal PG, Wu NL, Sun J et al (2010) Inactivation of Msx1 and Msx2 in neural crest reveals an unexpected role in suppressing heterotopic bone formation in the head. *Dev Biol* 343:28–39. <https://doi.org/10.1016/j.ydbio.2010.04.007>
- Li Z, Xu Z, Duan C et al (2018) Role of TCF/LEF transcription factors in bone development and osteogenesis. *Int J Med Sci* 15(12):1415–1422. <https://doi.org/10.7150/ijms26741>
- Kyllönen L, Haimi S, Mannerström B et al (2013) Effects of different serum conditions on osteogenic differentiation of human adipose stem cells in vitro. *Stem Cell Res Ther* 4(1):17. <https://doi.org/10.1186/srct165>

33. Huang Z, Nelson ER, Smith RL, Goodman SB (2007) The sequential expression profiles of growth factors from osteoprogenitors [correction of osteoprogenitors] to osteoblasts in vitro. *Tissue Eng* 13:2311–2320
34. Quarles LD, Yohay DA, Lever LW et al (1992) Distinct proliferative and differentiated stages of murine MC3T3-E1 cells in culture: an in vitro model of osteoblast development. *J Bone Miner Res* 7:683–692
35. Hoemann CD, El-Gabalawy H, McKee MD (2009) In vitro osteogenesis assays: influence of the primary cell source on alkaline phosphatase activity and mineralization. *Pathol Biol* 57:318–323. <https://doi.org/10.1016/j.patbio.2008.06.004>
36. Bates P, Ramachandran M (2007) Bone injury, healing and grafting. In: Ramachandran M (ed) *Basic orthopaedic sciences. The Stanmore Guide*. Hodder Arnold, London, pp 123–134
37. Jones E, English A, Churchman SM et al (2002) Isolation and characterization of bone marrow multipotential mesenchymal progenitor cells. *Arthritis Rheum* 46(12):3349–3360
38. Bragdon BC, Bahney CS (2018) Origin of reparative stem cells in fracture healing. *Curr Osteoporos Rep* 6(4):490–503. <https://doi.org/10.1007/s11914-018-0458-4>
39. Almubarak S, Nethercott H, Freeberg M et al (2016) Tissue engineering strategies for promoting vascularized bone regeneration. *Bone* 83:197–209. <https://doi.org/10.1016/j.bone.2015.11.011>
40. Yu J, Vodyanik MA, Smuga-Otto K et al (2007) Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318:1917–1920
41. Ben-David U, Benvenisty N (2011) The tumorigenicity of human embryonic and induced pluripotent stem cells. *Nat Rev Cancer* 11:268–277. <https://doi.org/10.1038/nrc3034>
42. Gutierrez-Aranda I, Ramos-Mejia V, Bueno C et al (2010) Human induced pluripotent stem cells develop teratoma more efficiently and faster than human embryonic stem cells regardless the site of injection. *Stem Cells* 28:1568–1570. <https://doi.org/10.1002/stem471>
43. Friedenstein AJ, Chailakhyan RK, Latsinik NV et al (1974) Stromal cells responsible for transfer-ring the microenvironment of the hemopoietic tissues cloning in vitro and retransplantation in vivo. *Transplantation* 17:331–340
44. Kang NH, Hwang KA, Kim SU et al (2012) Potential antitumor therapeutic strategies of human amniotic membrane and amniotic fluid-derived stem cells. *Cancer Gene Ther* 19(8):517–522. <https://doi.org/10.1038/cgt201230>
45. Prockop DJ, Kota DJ, Bazhanov N, Reger RL (2010) Evolving paradigms for repair of tissues by adult stem/progenitor cells (MSCs). *J Cell Mol Med* 14:2190–2199. <https://doi.org/10.3109/146532492010507330>
46. Pountos I, Georgouli T, Kontakis G, Giannoudis PV (2010) Efficacy of minimally invasive techniques for enhancement of fracture healing: evidence today. *Int Orthop* 34(1):3–12. <https://doi.org/10.1007/s00264-009-0892-0>
47. Augello A, Kurth TB, De Bari C (2010) Mesenchymal stem cells: a perspective from in vitro cultures to in vivo migration and niches. *Eur Cell Mater* 20:121–133
48. Alvarez-Buylla A, Garcia-Verdugo JM (2002) Neurogenesis in adult subventricular zone. *J Neurosci* 22:629–634. <https://doi.org/10.1523/jneurosci.22-03-00629.2002>
49. Nakagomi T, Takagi T, Beppu M et al (2019) Neural regeneration by regionally induced stem cells within post-stroke brains: novel therapy perspectives for stroke patients. *World J Stem Cells* 11(8):452–463. <https://doi.org/10.4252/wjscv11i8452>
50. Jiang Y, Jahagirdar BN, Reinhardt RL et al (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 418:41–49
51. Tremain N, Korkko J, Ibberson D et al (2001) MicroSAGE analysis of 2353 expressed genes in a single cell-derived colony of undifferentiated human mesenchymal stem cells reveals mRNAs of multiple cell lineages. *Stem Cells* 19:408–418
52. Schwartz RE, Reyes M, Koodie L et al (2002) Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. *J Clin Invest* 109:1291–1302
53. Tropel P, Platet N, Platel JC et al (2006) Functional neuronal differentiation of bone marrow-derived mesenchymal stem cells. *Stem Cells* 24:2868–2876
54. Rose RA, Keating A, Backx PH (2008) Do mesenchymal stromal cells transdifferentiate into functional cardiomyocytes? *Circ Res* 103:e120. <https://doi.org/10.1161/CIRCRESAHA.108186908>
55. Pijnappels DA, Schaliij MJ, Ramkisoensing AA et al (2008) Forced alignment of mesenchymal stem cells undergoing cardiomyogenic differentiation affects functional integration with cardiomyocyte cultures. *Circ Res* 103:167–176. <https://doi.org/10.1161/CIRCRESAHA.108176131>
56. Bhattacharyya S, Kumar A, Lal Khanduja K (2012) The voyage of stem cell toward terminal differentiation: a brief overview. *Acta Biochim Biophys Sin (Shanghai)* 4(6):463–475. <https://doi.org/10.1093/abbs/gms027>
57. Dominici M, Le Blanc K, Mueller I et al (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8(4):315–317
58. Tokalov SV, Grüner S, Schindler S et al (2007) Age-related changes in the frequency of mesenchymal stem cells in the bone marrow of rats. *Stem Cells Dev* 16(3):439–446
59. Dalton S, Smith K, Singh K et al (2020) Accumulation of kynurenine elevates oxidative stress and alters microRNA profile in human bone marrow stromal cells. *Exp Gerontol* 130:110800. <https://doi.org/10.1016/j.exger.2019.110800>
60. Li S, Wang Y, Guan L, Ji M (2015) Characteristics of human umbilical cord mesenchymal stem cells during ex vivo expansion. *Mol Med Rep* 12:4320–4325. <https://doi.org/10.3892/mmr.2015.3999>
61. Araújo AB, Salton GD, Furlan JM et al (2017) Comparison of human mesenchymal stromal cells from four neonatal tissues: amniotic membrane, chorionic membrane, placental decidua and umbilical cord. *Cytotherapy* 19(5):577–585. <https://doi.org/10.1016/j.jcyt.2017.03.001>
62. Moraghebi R, Kirkeby A, Chaves P et al (2017) Term amniotic fluid: an unexploited reserve of mesenchymal stromal cells for reprogramming and potential cell therapy applications. *Stem Cell Res Ther* 8(1):190. <https://doi.org/10.1186/s13287-017-0582-6>
63. Bajek A, Olkowska J, Walentowicz-Sadlecka M et al (2017) High quality independent from a donor: human amniotic fluid derived stem cells—a practical analysis based on 165 clinical cases. *J Cell Biochem* 118(1):116–126. <https://doi.org/10.1002/jcb.25618>
64. Liu J, Yu F, Sun Y (2015) Concise reviews: characteristics and potential applications of human dental tissue-derived mesenchymal stem cells. *Stem Cells* 33(3):627–638. <https://doi.org/10.1002/stem1909>
65. Botelho J, Cavacas MA, Machado V, Mendes JJ (2017) Dental stem cells: recent progresses in tissue engineering and regenerative medicine. *Ann Med* 49(8):644–651. <https://doi.org/10.1080/0785389020171347705>
66. Zuk PA, Zhu M, Mizuno H et al (2001) Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng* 7(2):211–228
67. Zimmerlin L, Donnenberg VS, Pfeifer ME et al (2010) Stromal vascular progenitors in adult human adipose tissue. *Cytometry A* 77(1):22–30. <https://doi.org/10.1002/cytoa.20813>
68. Bourin P, Bunnell BA, Casteilla L et al (2013) Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint

- statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy* 15(6):641–648. <https://doi.org/10.1016/j.jcyt.2013.02.006>
69. Dufrene D (2017) Impact of age on human adipose stem cells for bone tissue engineering. *Cell Transplant* 26:1496–1504. <https://doi.org/10.1177/0963689717721203>
 70. D'Alimonte I, Mastrangelo F, Giuliani P et al (2017) Osteogenic differentiation of mesenchymal stromal cells: a comparative analysis between human subcutaneous adipose tissue and dental pulp. *Stem Cells Dev* 26(11):843–855. <https://doi.org/10.1089/scd.2016.0190>
 71. Strem BM, Hicok KC, Zhu M et al (2005) Multipotential differentiation of adipose tissue-derived stem cells. *Keio J Med* 54(3):132–141
 72. Kern S, Eichler H, Stoeve J et al (2006) Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells* 24(5):1294–1301
 73. Banyard DA, Salibian AA, Widgerow AD, Evans GR (2015) Implications for human adipose-derived stem cells in plastic surgery. *J Cell Mol Med* 19(1):21–30. <https://doi.org/10.1111/jcmm.12425>
 74. Frese L, Dijkman PE, Hoerstrup SP (2016) Adipose tissue-derived stem cells in regenerative medicine. *Transfus Med Hemother* 43(4):268–274
 75. Li J, Martin EC, Gimble JM (2016) Adipose-derived stem/stromal cells, in: regenerative medicine—from protocol to patient 2. *Stem Cell Science and Technology*. G Steinhoff. Springer International Publishing AG Switzerland pp 363–387.
 76. Virjula S, Zhao F, Leivo J et al (2017) The effect of equiaxial stretching on the osteogenic differentiation and mechanical properties of human adipose stem cells. *J Mech Behav Biomed Mater* 72:38–48. <https://doi.org/10.1016/j.jmbbm.2017.04.016>
 77. Li Z, Liu C, Xie Z et al (2011) Epigenetic dysregulation in mesenchymal stem cell aging and spontaneous differentiation. *PLoS One* 6(6):e20526. <https://doi.org/10.1371/journal.pone.0020526>
 78. Jackson WM, Nesti LJ, Tuan RS (2012) Concise review: clinical translational of wound healing therapies based on mesenchymal stem cells. *Stem Cells. Translat Med* 1:44–50. <https://doi.org/10.5966/sctm.2011-0024>
 79. Liao HT, Chen CT (2014) Osteogenic potential: Comparison between bone marrow and adipose-derived mesenchymal stem cells. *World J Stem Cells* 6(3):288–295. <https://doi.org/10.4252/wjscv.6i3288>
 80. Han Y, Li X, Zhang Y et al (2019) Mesenchymal stem cells for regenerative medicine. *Cells* 8(8):pii: E886. <https://doi.org/10.3390/cells8080886>
 81. Yorukoglu AC, Kiter AE, Akkaya S et al (2017, 2017) A concise review on the use of mesenchymal stem cells in cell s-based tissue engineering with special emphasis on bone tissue regeneration. *Stem Cells Int*:2374161. <https://doi.org/10.1155/2017/2374161>
 82. Soleimanifar F, Hosseini FS, Atabati H et al (2019) Adipose-derived stem cells-conditioned medium improved osteogenic differentiation of induced pluripotent stem cells when grown on polycaprolactone nanofibers. *J Cell Physiol* 234(7):10315–10323. <https://doi.org/10.1002/jcp.27697>
 83. Ishihara A, Bertone AL (2012) Cell-mediated and direct gene therapy for bone regeneration. *Expert Opin Biol Ther* 12:411–423. <https://doi.org/10.1517/147125982012661709>
 84. Evans NR, Davies EM, Dare CJ, Oreffo RO (2013) Tissue engineering strategies in spinal arthrodesis: the clinical imperative and challenges to clinical translation. *Regen Med* 8(1):49–64. <https://doi.org/10.2217/rme.12106>
 85. Dhulakhandi DB, Rohilla S, Rattan KN (2010) Neural tube defects: review of experimental evidence on stem cell therapy and newer treatment options. *Fetal Diagn Ther* 28:72–78. <https://doi.org/10.1159/000318201>
 86. Li H, Gao F, Ma L et al (2012) Therapeutic potential of in utero mesenchymal stem cell (MSCs) transplantation in rat fetuses with spina bifida aperta. *J Cell Mol Med* 16:1606–1617. <https://doi.org/10.1111/j.1582-4934.2011.01470x>
 87. Steigman SA, Ahmed A, Shanti RM et al (2009) Sternal repair with bone grafts engineered from amniotic mesenchymal stem cells. *J Pediatr Surg* 44:1120–1126. <https://doi.org/10.1016/j.jpedsurg.2009.02.038>
 88. Duailibi MT, Duailibi SE, Youn CS et al (2004) Bioengineered teeth from cultured rat tooth bud cells. *J Dent Res* 83:523–528
 89. Young CS, Abukawa H, Asrican R et al (2005) Tissue-engineered hybrid tooth and bone. *Tissue Eng* 11:1599–1610
 90. Shanbhag S, Pandis N, Mustafa K et al (2017) Alveolar bone tissue engineering in critical-size defects of experimental animal models: a systematic review and meta-analysis. *J Tissue Eng Regen Med* 11(10):2935–2949. <https://doi.org/10.1002/term.2198>
 91. Monsarrat P, Vergnes JN, Nabet C et al (2014) Concise review: mesenchymal stromal cells used for periodontal regeneration: a systematic review. *Stem Cells Transl Med Jun* 3(6):768–774. <https://doi.org/10.5966/sctm.2013-0183>
 92. Trofin EA, Monsarrat P, Kemoun P (2013) Cell therapy of periodontium: from animal to human? *Front Physiol* 4:325. <https://doi.org/10.3389/fphys.2013.00325>
 93. Volk SW, Theoret C (2013) Translating stem cell therapies: the role of companion animals in regenerative medicine. *Wound Rep Reg* 21:382–394. <https://doi.org/10.1111/wrr.12044>
 94. Khojasteh A, Behnia H, Dashti SG, Stevens M (2012) Current trends in mesenchymal stem cell application in bone augmentation: a review of the literature. *J Oral Maxillofac Surg* 70(4):972–982. <https://doi.org/10.1016/j.joms.2011.02.133>
 95. Janicki P, Schmidmaier G (2011) What should be the characteristics of the ideal bone graft substitute? Combining scaffolds with growth factors and/or stem cells. *Injury Int J* 42:577–581. <https://doi.org/10.1016/j.injury.2011.06.014>
 96. Lieberman JR, Daluiski A, Einhorn TA (2002) The role of growth factors in the repair of bone. Biology and clinical applications. *J Bone Joint Surg Am* 84A(6):1032–1044
 97. Quarto R, Mastrogiacomo M, Cancedda R et al (2001) Repair of large bone defects with the use of autologous bone marrow stromal cells. *N Engl J Med* 344(5):385–386
 98. Hernigou P, Poignard A, Beaujean F, Rouard H (2005) Percutaneous autologous bone-marrow grafting for non-unions. Influence of the number and concentration of progenitor cells. *J Bone Joint Surg Am* 87(7):1430–1437
 99. Marcacci M, Kon E, Moukhachev V et al (2007) Stem cells associated with macro-porous bioceramics for long bone repair: 6- to 7-year outcome of a pilot clinical study. *Tissue Eng* 13(5):947–955
 100. Šponer P, Kučera T, Brtková J et al (2018) Comparative study on the application of mesenchymal stromal cells combined with tricalcium phosphate scaffold into femoral bone defects. *Cell Transplant* 27(10):1459–1468. <https://doi.org/10.1177/0963689718794918>
 101. Hernigou P, Dubory A, Homma Y et al (2018) Cell therapy versus simultaneous contralateral decompression in symptomatic corticosteroid osteonecrosis: a thirty-year follow-up prospective randomized study of one hundred and twenty-five adult patients. *Int Orthop* 42(7):1639–1649. <https://doi.org/10.1007/s00264-018-3941-8>
 102. Noriega DC, Ardura F, Hernández-Ramajo R et al (2017) Intervertebral disc repair by allogeneic mesenchymal bone marrow cells: a randomized controlled trial. *Transplantation* 101(8):1945–1951. <https://doi.org/10.1097/TP.0000000000001484>

103. Gómez-Barrena E, Rosset P, Lozano D et al (2015) Bone fracture healing: cell therapy in delayed unions and nonunions. *Bone* 70: 93–101. <https://doi.org/10.1016/j.bone.2014.07.033>
104. Cristante AF, Barros-Filho TE, Tatsui N et al (2009) Stem cells in the treatment of chronic spinal cord injury: evaluation of somatosensitive evoked potentials in 39 patients. *Spinal Cord* 47:733–738. <https://doi.org/10.1038/sc.2009.24>
105. Karamouzian S, Nematollahi-Mahani SN, Nakhaee N, Eskandary H (2012) Clinical safety and primary efficacy of bone marrow mesenchymal cell trans-plantation in subacute spinal cord injured patients. *Clin Neurol Neurosurg* 114:935–939. <https://doi.org/10.1038/cgt.2012.30>
106. Forostyak S, Jendelova P, Sykova E (2013) The role of mesenchymal stromal cells in spinal cord injury, regenerative medicine and possible clinical applications. *Biochimie* 95:2257–2270. <https://doi.org/10.1016/j.biochi.2013.08.004>
107. Gjerde C, Mustafa K, Hellem S et al (2018) Cell therapy induced regeneration of severely atrophied mandibular bone in a clinical trial. *Stem Cell Res Ther* 9(1):213. <https://doi.org/10.1186/s13287-018-0951-9>
108. Egusa H, Sonoyama W, Nishimura M et al (2012) Stem cells in dentistry—part I: clinical applications. *J Prosthodont Res* 56:229–248. <https://doi.org/10.1016/j.jpor.2012.10.001>
109. Egusa H, Sonoyama W, Nishimura M et al (2012) Stem cells in dentistry—part II: clinical applications. *J Prosthodont Res* 56:229–248. <https://doi.org/10.1016/j.jpor.2012.10.001>
110. Norambuena GA, Khoury M, Jorgensen C (2012) Mesenchymal stem cells in osteoarticular pediatric diseases: an update. *Pediatric Res* 71(4):452–458. <https://doi.org/10.1038/pr.2011.68>
111. Jin SE, Sung JH (2016) Hair regeneration using adipose-derived stem cells. *Histol Histopathol* 31(3):249–256. <https://doi.org/10.14670/HH-11-686>
112. Di Summa PG, Kingham PJ, Raffoul W et al (2010) Adipose-derived stem cells enhance peripheral nerve regeneration. *J Plast Reconstr Aesthet Surg* 63(9):1544–1552. <https://doi.org/10.1016/j.jbjs.2009.09.012>
113. Brenner CH, David R, Franz WM (2016) Stem cells for cardiovascular regeneration. In: *Regenerative Medicine – from Protocol to Patient*, 2. Springer International Publishing AG Switzerland, Stem Cell Science and Technology. G Steinhoff, pp 145–167
114. Lin G, Wang G, Liu G et al (2009) Treatment of type 1 diabetes with adipose tissue-derived stem cells expressing pancreatic duodenal homeobox 1. *Stem Cells Dev* 18(10):1399–1406. <https://doi.org/10.1089/scd.2009.0010>
115. Banas A, Teratani T, Yamamoto Y et al (2009) Rapid hepatic fate specification of adipose-derived stem cells and their therapeutic potential for liver failure. *J Gastroenterol Hepatol* 24(1):70–77. <https://doi.org/10.1111/j.1440-1746.2008.05496.x>
116. Collawn SS, Banerjee NS, De La Torre J et al (2012) Adipose-derived-stromal cells accelerate wound healing in an organotypic raft culture model. *Ann Plast Surg* 68(5):501–504. <https://doi.org/10.1097/SAP.0b013e31823b69fc>
117. Strong AL, Bowles AC, Maccrimmon CP et al (2015) Adipose stromal cells repair pressure ulcers in both young and elderly mice: potential role of adipogenesis in skin repair. *Stem Cells Transl Med* 4(6):632–642. <https://doi.org/10.5966/sctm.2014-0235>
118. Lendeckel S, Jödicke A, Christophis P et al (2004) Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: case report. *J Craniomaxillofac Surg* 32(6): 370–373
119. Pak J, Lee JH, Park KS et al (2017) Current use of autologous adipose tissue-derived stromal vascular fraction cells for orthopedic applications. *J Biomed Sci* 24(1):9. <https://doi.org/10.1186/s12929-017-0318-z>
120. Merfeld-Clauss S, Gollahalli N, March KL, Traktuev DO (2010) Adipose tissue progenitor cells directly interact with endothelial cells to induce vascular network formation. *Tissue Eng. Part A* 16(9):2953–2966. <https://doi.org/10.1089/ten.TEA.2009.0635>
121. Veronesi F, Borsari V, Sartori M et al (2018) The use of cell conditioned medium for musculoskeletal tissue regeneration. *J Cell Physiol* 233(6):4423–4442. <https://doi.org/10.1002/jcp.26291>
122. Majidinia M, Sadeghpour A, Yousefi B (2018) The roles of signaling pathways in bone repair and regeneration. *J Cell Physiol* 233(4):2937–2948. <https://doi.org/10.1002/jcp.26042>
123. Burnstock G (2041) Introduction to Purinergic Signaling. *Methods Mol Biol* 2020:1–15. https://doi.org/10.1007/978-1-4939-9717-6_1
124. Brown W, Foote CS (1998) *Organic Chemistry*, 2nd Edition.
125. Camici M, Garcia-Gil M, Tozzi MG (2018) The inside story of adenosine. *Int J Mol Sci* 19(3):pii: E784. <https://doi.org/10.3390/ijms19030784>
126. Rathbone MP, Middlemiss PJ, Gysbers JW et al (1999) Trophic effects of purines in neurons and glial cells. *Prog. Neurobiol.* 59(6):663–690
127. Lazarowski ER, Sesma JJ, Seminario-Vidal L, Kreda SM (2011) Molecular mechanisms of purine and pyrimidine nucleotide release. *Adv Pharmacol* 61:221–261. <https://doi.org/10.1016/B978-0-12-385526-8.00008-4>
128. Burnstock G, Verkhratsky A (2012) Mechanisms of ATP release and inactivation. In: Burnstock G, Verkhratsky A (eds) *Purinergic signalling and the nervous system*. Germany. Springer, Berlin, pp 79–118
129. Ferrari D, McNamee EN, Idzko M, Gambari R et al (2016) Purinergic signaling during immune cell trafficking. *Trends in Immunology* 37:399–411. <https://doi.org/10.1016/j.it.2016.04.004>
130. Kong W, Engel K, Wang J (2004) Mammalian nucleoside transporters. *Curr Drug Metab* 5:63–84
131. Yegutkin GG (2008) Nucleotide- and nucleoside-converting ectoenzymes: important modulators of purinergic signalling cascade. *Biochim Biophys Acta* 1783:673–694. <https://doi.org/10.1016/j.bbamcr.2008.01.024>
132. Di Liberto V, Mudò G, Garozzo R et al (2016) The guanine-based purinergic system: the tale of an orphan neuromodulation. *Front Pharmacol* 7:158. <https://doi.org/10.3389/fphar.2016.00158>
133. Yegutkin GG (2014) Enzymes involved in metabolism of extracellular nucleotides and nucleosides: functional implications and measurement of activities. *Crit Rev Biochem Mol Biol* 49:473–497. <https://doi.org/10.3109/10409238.2014.953627>
134. Inoue K (2017) Molecular basis of nucleobase transport systems in mammals. *Biol Pharm Bull* 40(8):1130–1138. <https://doi.org/10.1248/bpb.b17-00374>
135. Glaser T, Cappellari AR, Pillat MM et al (2012) Perspectives of purinergic signaling in stem cell differentiation and tissue regeneration. *Purinergic Signal* 8(3):523–537. <https://doi.org/10.1007/s11302-011-9282-3>
136. Burnstock G (2014) Purinergic signalling: from discovery to current developments. *Exp Physiol* 99:16–34. <https://doi.org/10.1113/expphysiol.2013.071951>
137. Rafehi M, Müller CE (2018) Tools and drugs for uracil nucleotide-activated P2Y receptors. *Pharmacol Ther* 190:24–80. <https://doi.org/10.1016/j.pharmthera.2018.04.002>
138. Volonté C, D'Ambrosi N (2009) Membrane compartments and purinergic signalling: the purinome, a complex interplay among ligands, degrading enzymes, receptors and transporters. *FEBS J* 276(2):318–329. <https://doi.org/10.1111/j.1742-4658.2008.06793.x>
139. Di Virgilio F, Adinolfi E (2017) Extracellular purines, purinergic receptors and tumor growth. *Oncogene* 36(3):293–303. <https://doi.org/10.1038/ncr.2016.206>

140. Bakopoulou A, Apatzidou D, Aggelidou E et al (2017) Isolation and prolonged expansion of oral mesenchymal stem cells under clinical-grade, GMP-compliant conditions differentially affect “stemness” properties. *Stem Cell Res Ther* 8(1):247. <https://doi.org/10.1186/s13287-017-0705-0>
141. Genetos DC, Geist DJ, Liu D et al (2005) Fluid shear-induced ATP secretion mediates prostaglandin release in MC3T3-E1 osteoblasts. *J Bone Miner Res* 20:41–49
142. Romanello M, Codognotto A, Bicego M et al (2005) Autocrine/paracrine stimulation of purinergic receptors in osteoblasts: Contribution of vesicular ATP release. *Biochem Biophys Res Commun* 331:1429–1438
143. Orriss IR, Knight GE, Utting JC et al (2009) Hypoxia stimulates vesicular ATP release from rat osteoblasts. *J Cell Physiol* 220:155–162. <https://doi.org/10.1002/jcp.21745>
144. Sabirov RZ, Okada Y (2009) The maxi-anion channel: a classical channel playing novel roles through an unidentified molecular entity. *J Physiol Sci* 59:3–21. <https://doi.org/10.1007/s12576-008-0008-4>
145. Thi MM, Islam S, Suadcani SO, Spray DC (2012) Connexin43 and pannexin1 channels in osteoblasts: Who is the “hemichannel”? *J Membr Biol* 245: 401–409. <https://doi.org/10.1007/s00232-012-9462-2>
146. Brandao-Burch A, Key ML, Patel JJ et al (2012) The P2X7 Receptor is an important regulator of extracellular ATP levels. *Front Endocrinol (Lausanne)* 3:41. <https://doi.org/10.3389/fendo.2012.00041>
147. Donahue HJ (2000) Gap junctions and biophysical regulation of bone cell differentiation. *Bone* 26:417–422
148. Noronha-Matos JB, Costa MA, Magalhães-Cardoso MT et al (2012) Role of ecto-NTPDases on UDP-sensitive P2Y receptor activation during osteogenic differentiation of primary bone marrow stromal cells from postmenopausal women. *J Cell Physiol* 227:2694–2709. <https://doi.org/10.1002/jcp.23014>
149. Hecht E, Liedert A, Ignatius A et al (2013) Local detection of mechanically induced ATP release from bone cells with ATP microbiosensors. *Biosens Bioelectron* 44:27–33. <https://doi.org/10.1016/j.bios.2013.01.008>
150. Alvarenga EC, Rodrigues R, Caricati-Neto A et al (2010) Low-intensity pulsed ultrasound-dependent osteoblast proliferation occurs by via activation of the P2Y receptor: Role of the P2Y1 receptor. *Bone* 46:355–362. <https://doi.org/10.1016/j.bone.2009.09.017>
151. Burnstock G, Arnett TR, Orriss IR et al (2013) Purinergic signaling in the musculo-skeletal system. *Purinergic Signal* 9:541–572. <https://doi.org/10.1007/s11302-013-9381-4>
152. Roszek K, Błaszczak A, Wujak M, Komoszyński M (2013) Nucleotides metabolizing ecto-enzymes as possible markers of mesenchymal stem cell osteogenic differentiation. *Biochem Cell Biol* 91:176–181. <https://doi.org/10.1139/bcb-2012-0093>
153. Roszek K, Bomastek K, Drożdżal M, Komoszyński M (2013) Dramatic differences in activity of purines metabolizing ecto-enzymes between mesenchymal stem cells isolated from human umbilical cord blood and umbilical cord tissue. *Biochem Cell Biol* 91:519–525. <https://doi.org/10.1139/bcb-2013-0050>
154. Iser IC, Bracco PA, Gonçalves CEI et al (2014) Mesenchymal stem cells from different murine tissues have differential capacity to metabolize extracellular nucleotides. *J. Cell Biochem* 115:1673–1682. <https://doi.org/10.1002/jcb.24830>
155. Naasani LIS, Rodrigues C, de Campos RP et al (2017) Extracellular nucleotide hydrolysis in dermal and limbal mesenchymal stem cells: a source of adenosine production. *J. Cell Biochem* 118:2430–2442. <https://doi.org/10.1002/jcb.25909>
156. Katebi M, Soleimani M, Cronstein BN (2009) Adenosine A2A receptors play an active role in mouse bone marrow-derived mesenchymal stem cell development. *J Leukoc Biol* 85:438–444. <https://doi.org/10.1189/jlb.0908520>
157. Hoebertz A, Arnett TR, Burnstock G (2003) Regulation of bone resorption and formation by purines and pyrimidines. *Trends Pharmacol Sci.* 24(6):290–297
158. Zhang B, Hou R, Zou Z, Luo T, Zhang Y, Wang L, Wang B (2018) Mechanically induced autophagy is associated with ATP metabolism and cellular viability in osteocytes in vitro. *Redox Biol* 14:492–498. <https://doi.org/10.1016/j.redox.2017.10.021>
159. Ferrari D, Gulinelli S, Salvestrini V et al (2011) Purinergic stimulation of human mesenchymal stem cells potentiates their chemotactic response to CXCL12 and increases the homing capacity and production of proinflammatory cytokines. *Exp Hematol* 39:360–374. <https://doi.org/10.1016/j.exphem.2010.12.001>
160. Peng H, Hao Y, Mousawi F, Roger S, Li J, Sim JA, Jiang LH (2016) Purinergic and store-operated Ca²⁺ signalling mechanisms in mesenchymal stem cells and their roles in ATP-induced stimulation of cell migration. *Stem Cells* 34:2102–2114. <https://doi.org/10.1002/stem.2370>
161. Evans BA, Elford C, Pexa A et al (2006) Human osteoblast precursors produce extracellular adenosine, which modulates their secretion of IL-6 and osteoprotegerin. *J Bone Miner Res* 21:228–236. <https://doi.org/10.1359/JBMR.051021>
162. Costa MA, Barbosa A, Neto E et al (2011) On the role of subtype selective adenosine receptor agonists during proliferation and osteogenic differentiation of human primary bone marrow stromal cells. *J Cell Physiol* 226(5):1353–1366. <https://doi.org/10.1002/jcp.22458>
163. Tuli R, Tuli S, Nandi S et al (2003) Characterization of multipotential mesenchymal progenitor cells derived from human trabecular bone. *Stem Cells* 21:681–693
164. Hau K-L, Ranzoni AM, Vlahova F et al (2017) TGFβ-induced osteogenic potential of human amniotic fluid stem cells via CD73-generated adenosine production. *Scientific Reports* 7:6601. <https://doi.org/10.1038/s41598-017-06780-1>
165. Lenertz LY, Baughman CJ, Waldschmidt NV et al (2015) Control of bone development by P2X and P2Y receptors expressed in mesenchymal and hematopoietic cells. *Gene* 570(1):1–7. <https://doi.org/10.1016/j.gene.2015.06.031>
166. Orriss IR, Burnstock G, Arnett TR (2010) Purinergic signalling and bone remodelling. *Curr Opin Pharmacol* 10(3):322–330. <https://doi.org/10.1016/j.coph.2010.01.003>
167. He W, Cronstein BN (2012) Adenosine A1 receptor regulates osteoclast formation by altering TRAF6/TAK1 signaling. *Purinergic Signal* 8(2):327–337. <https://doi.org/10.1007/s11302-012-9292-9>
168. Mediero A, Perez-Aso M, Cronstein BN (2013) Activation of adenosine A2A receptor reduces osteoclast formation via PKA and ERK1/2-mediated suppression of NFκB nuclear translocation. *Br J Pharmacol* 169(6):1372–1388. <https://doi.org/10.1111/bph.12227>
169. Mediero A, Kara FM, Wilder T, Cronstein BN (2012) Adenosine A2A receptor ligation inhibits osteoclast formation. *Am J Pathol* 180(2):775–786. <https://doi.org/10.1016/j.ajpath.2011.10.017>
170. Vaupel P, Mayer A (2016) Hypoxia-driven adenosine accumulation: a crucial microenvironmental factor promoting tumor progression. *Adv Exp Med Biol* 876:177–183. https://doi.org/10.1007/978-1-4939-3023-4_22
171. Borhani S, Corciulo C, Larranaga-Vera A, Cronstein BN (2019) Adenosine A(2A) receptor (A2AR) activation triggers Akt signaling and enhances nuclear localization of β-catenin in osteoblasts. *FASEB J* 33(6):7555–7562. <https://doi.org/10.1096/fj.201900014R>
172. He W, Mazumder A, Wilder T, Cronstein BN (2013) Adenosine regulates bone metabolism via A1, A2A, and A2B receptors in bone marrow cells from normal humans and patients with multiple

- myeloma. *FASEB J.* 27(9):3446–3454. <https://doi.org/10.1096/fj.13-231233>
173. Gharibi B, Abraham AA, Ham J, Evans BA (2011) Adenosine receptor subtype expression and activation influence the differentiation of mesenchymal stem cells to osteoblasts and adipocytes. *J Bone Miner Res* 26:2112–2124. <https://doi.org/10.1002/jbmr.424>
 174. Carroll SH, Wigner NA, Kulkarni N et al (2012) A2B adenosine receptor promotes mesenchymal stem cell differentiation to osteoblasts and bone formation in vivo. *J Biol Chem* 287:15718–15727. <https://doi.org/10.1074/jbc.M112.344994>
 175. Ciciarello M, Zini R, Rossi L et al (2013) Extracellular purines promote the differentiation of human bone marrow-derived mesenchymal stem cells to the osteogenic and adipogenic lineages. *Stem Cells Dev* 22:1097–1111. <https://doi.org/10.1089/scd.2012.0432>
 176. Hajjawi MOR, Patel JJ, Corcelli M et al (2016) Lack of effect of adenosine on the function of rodent osteoblasts and osteoclasts in vitro. *Purinergic Signal* 12(2):247–258. <https://doi.org/10.1007/s11302-016-9499-2>
 177. Rath-Wolfson L, Bar-Yehuda S, Madi L et al (2006) IBMECA, an A3 adenosine receptor agonist prevents bone resorption in rats with adjuvant induced arthritis. *Clin Exp Rheumatol* 24(4):400–406
 178. Orriss IR, Key ML, Brandao-Burch A et al (2012) The regulation of osteoblast function and bone mineralisation by extracellular nucleotides: the role of p2x receptors. *Bone* 51:389–400. <https://doi.org/10.1016/j.bone.2012.06.013>
 179. Carluccio M, Zuccarini M, Ziberi S et al (2019) Involvement of P2X7 receptors in the osteogenic differentiation of mesenchymal stromal/stem cells derived from human subcutaneous adipose tissue. *Stem Cell Rev* 15(4):574–589. <https://doi.org/10.1007/s12015-019-09883-6>
 180. Morrison MS, Turin L, King BF et al (1998) ATP is a potent stimulator of the activation and formation of rodent osteoclasts. *J Physiol* 511:495–500
 181. Kanaya K, Iba K, Dohke T, Okazaki S, Yamashita T (2016) TRPV1, ASICs and P2X2/3 expressed in bone cells simultaneously regulate bone metabolic markers in ovariectomized mice. *J Musculoskelet Neuronal Interact* 16(2):145–151
 182. Orriss IR, Knight GE, Ranasinghe S, Burnstock G, Arnett TR (2006) Osteoblast responses to nucleotides increase during differentiation. *Bone* 39(2):300–309
 183. Nakamura E, Uezono Y, Nurusawa K et al (2000) ATP activates DNA synthesis by acting on P2X receptors in human osteoblast-like MG-63 cells. *Am J Physiol Cell Physiol* 279:C510–C519
 184. Gartland A, Hipskind RA, Gallagher JA, Bowler WB (2001) Expression of a P2X7 receptor by a subpopulation of human osteoblasts. *J Bone Miner Res* 16:846–856
 185. Gartland A, Orriss IR, Rumney RM et al (2012) Purinergic signalling in osteoblasts. *Front Biosci* 17:16–29
 186. Agrawal A, Henriksen Z, Syberg S et al (2017) P2X7Rs are involved in cell death, growth and cellular signaling in primary human osteoblasts. *Bone* 95:101. <https://doi.org/10.1016/j.bone.2016.11.011>
 187. Noronha-Matos JB, Coimbra J, Sa-e-Sousa RR et al (2014) P2X7-induced zeiosis promotes osteogenic differentiation and mineralization of postmenopausal bone marrow-derived mesenchymal stem cells. *FASEB J* 28:5208–5222. <https://doi.org/10.1096/fj.14-257923>
 188. Rodrigues-Ribeiro R, Alvarenga EC, Calio ML et al (2015) Dual role of P2 receptors during osteoblast differentiation. *Cell Biochem Biophys* 71(2):1225–1233. <https://doi.org/10.1007/s12013-014-0332-7>
 189. Bowler WB, Dixon CJ, Halleux C et al (1999) Signaling in human osteoblasts by extracellular nucleotides. Their weak induction of the c-fos proto-oncogene via Ca²⁺ mobilization is strongly potentiated by a parathyroid hormone/cAMP-dependent protein kinase pathway independently of mitogen-activated protein kinase. *J Biol Chem* 274:14315–14324
 190. Hoebertz A, Mahendran S, Burnstock G, Arnett TR (2002) ATP and UTP at low concentrations strongly inhibit bone formation by osteoblasts: a novel role for the P2Y2 receptor in bone remodeling. *J Cell Biochem* 86:413–419
 191. Li W, Wei S, Liu C et al (2016) Regulation of the osteogenic and adipogenic differentiation of bone marrow-derived stromal cells by extracellular uridine triphosphate: the role of P2Y2 receptor and ERK1/2 signaling. *Int J Mol Med* 37(1):63–73. <https://doi.org/10.3892/ijmm.2015.2400>
 192. Liu PS, Chen CY (2010) Butyl benzyl phthalate suppresses the ATP-induced cell proliferation in human osteosarcoma HOS cells. *Toxicol Appl Pharmacol* 244:308–314. <https://doi.org/10.1016/j.taap.2010.01.007>
 193. Syberg S, Brandao-Burch A, Patel JJ et al (2012) Clopidogrel (Plavix), a P2Y12 receptor antagonist, inhibits bone cell function in vitro and decreases trabecular bone in vivo. *J Bone Miner Res* 27:2373–2386. <https://doi.org/10.1002/jbmr.1690>
 194. Biver G, Wang N, Gartland A et al (2013) Role of the P2Y13 receptor in the differentiation of bone marrow stromal cells into osteoblasts and adipocytes. *Stem Cells* 31:2747–2758. <https://doi.org/10.1002/stem.1411>
 195. Cho J, Yusuf R, Kook S et al (2014) Purinergic P2Y₁₄ receptor modulates stress-induced hematopoietic stem/progenitor cell senescence. *J Clin Invest* 124(7):3159–3171. <https://doi.org/10.1172/JCI61636>
 196. Zippel N, Limbach CA, Ratajski N et al (2012) Purinergic receptors influence the differentiation of human mesenchymal stem cells. *Stem Cells Dev* 21:884–900. <https://doi.org/10.1089/scd.2010.0576>
 197. Kotova PD, Bystrova MF, Rogachevskaja OA et al (2018) Coupling of P2Y receptors to Ca(2+) mobilization in mesenchymal stromal cells from the human adipose tissue. *Cell Calcium* 71: 1–14. <https://doi.org/10.1016/j.ceca.2017.11.001>
 198. Forostyak O, Butenko O, Anderova M et al (2016) Specific profiles of ion channels and ionotropic receptors define adipose- and bone marrow derived stromal cells. *Stem Cell Res* 16:622–634. <https://doi.org/10.1016/j.scr.2016.03.010>
 199. Ali S, Turner J, Fountain SJ (2018) P2Y(2) and P2Y(6) receptor activation elicits intracellular calcium responses in human adipose-derived mesenchymal stromal cells. *Purinergic Signal* 14(4):371–384. <https://doi.org/10.1007/s11302-018-9618-3>
 200. Strazzulla LC, Cronstein BN (2016) Regulation of bone and cartilage by adenosine signaling. *Purinergic Signal* 12:583–593
 201. Eijken M, Meijer IMJ, Westbroek I et al (2008) Wnt signaling acts and is regulated in a human osteoblast differentiation dependent manner. *J Cell Biochem* 104(2):568–579. <https://doi.org/10.1002/jcb.21651>
 202. Van der Horst G, van der Werf SM, Farih-Sips H et al (2005) Downregulation of Wnt signaling by increased expression of Dickkopf-1 and -2 is a prerequisite for late-stage osteoblast differentiation of KS483 cells. *J Bone Miner Res* 20(10):1867–1877
 203. Mediero A, Wilder T, Perez-Aso M, Cronstein BN (2015) Direct or indirect stimulation of adenosine A2A receptors enhances bone regeneration as well as bone morphogenetic protein-2. *FASEB J* 29:1577–1590. <https://doi.org/10.1096/fj.14-265066>
 204. Bronwen AJ, Evans BA (2012) Does adenosine play a role in bone formation, resorption and repair? *Purinergic Signal* 8(2): 177–180. <https://doi.org/10.1007/s11302-012-9317-4>
 205. Shih YV, Liu M, Knon SK et al (2019) Dysregulation of ectonucleotidase-mediated extracellular adenosine during postmenopausal bone loss. *Sci Adv* 5(8):eaax1387. <https://doi.org/10.1126/sciadv.aax1387>

206. Plotkin LI, Manolagas SC, Bellido T (2002) Transduction of cell survival signals by connexin-43 hemichannels. *J. Biol. Chem.* 277:8648–8657
207. Orriss IR, Guneri D, Hajjawi MOR et al (2017) Activation of the P2Y(2) receptor regulates bone cell function by enhancing ATP release. *J Endocrinol* 233:341–356. <https://doi.org/10.1530/JOE-17-0042>
208. Du D, Zhou Z, Zhu L et al (2018) TNF- α suppresses osteogenic differentiation of MSCs by accelerating P2Y(2) receptor in estrogen-deficiency induced osteoporosis. *Bone* 117:161–170. <https://doi.org/10.1016/j.bone.2018.09.012>
209. Alqallaf SM, Evans BA, Kidd EJ (2009) A typical P2X receptor pharmacology in two human osteoblast-like cell lines. *Br J Pharmacol* 156:1124–1135. <https://doi.org/10.1111/j.1476-5381.2009.00119.x>
210. Ihara H, Hirukawa K, Goto S, Togari A (2005) ATP-stimulated interleukin-6 synthesis through P2Y receptors on human osteoblasts. *Biochem Biophys Res Commun* 326:329–334
211. Panupinthu N, Rogers JT, Zhao L et al (2008) P2X7 receptors on osteoblasts couple to production of lysophosphatidic acid: a signaling axis promoting osteogenesis. *J Cell Biol* 181:859–871. <https://doi.org/10.1083/jcb.200708037>
212. Panupinthu N, Zhao L, Possmayer F et al (2007) P2X7 nucleotide receptors mediate blebbing in osteoblasts through a pathway involving lysophosphatidic acid. *J Biol Chem* 282:3403–3412
213. Li J, Liu D, Ke HZ et al (2005) The P2X7 nucleotide receptor mediates skeletal mechanotransduction. *J Biol Chem* 280:42952–42959
214. Ke HZ, Qi H, Weidema AF et al (2003) Deletion of the P2X7 nucleotide receptor reveals its regulatory roles in bone formation and resorption. *Mol Endocrinol* 17:1356–1367
215. Friedman E, Moran DS, Ben-Avraham D, Yanovich R, Atzmon G (2014) Novel candidate genes putatively involved in stress fracture predisposition detected by whole-exome sequencing. *Genet Res (Camb)* 96:e004. <https://doi.org/10.1017/S001667231400007X>
216. Jorgensen NR, Husted LB, Skarratt KK et al (2012) Single-nucleotide polymorphisms in the P2X7 receptor gene are associated with postmenopausal bone loss and vertebral fractures. *Eur J Hum Genet* 20:675–681. <https://doi.org/10.1038/ejhg.2011.253>
217. Ohlendorff SD, Tofteng CL, Jensen JE et al (2007) Single nucleotide polymorphisms in the P2X7 gene are associated to fracture risk and to effect of estrogen treatment. *Pharmacogenet Genomics* 17:555–567
218. Husted LB, Harslof T, Stenkjaer L et al (2013) Functional polymorphisms in the P2X7 receptor gene are associated with osteoporosis. *Osteoporos Int* 24:949–959. <https://doi.org/10.1007/s00198-012-2035-5>
219. Varley I, Greeves JP, Sale C et al (2016) Functional polymorphisms in the P2X7 receptor gene are associated with stress fracture injury. *Purinergic Signal* 12(1):103–113. <https://doi.org/10.1007/s11302-016-9495-6>
220. Gharibi B, Abraham AA, Ham J, Evans BA (2012) Contrasting effects of A1 and A2b adenosine receptors on adipogenesis. *Int J Obes* 36:397–406
221. North RA (2002) Molecular physiology of P2X receptors. *Physiol Rev* 82:1013–1067
222. Sperl agh B, Illes P (2014) P2X7 receptor: an emerging target in central nervous system diseases. *Trends Pharmacol Sci* 35(10):537–547. <https://doi.org/10.1016/j.tips.2014.08.002>
223. Cheewatrakoolpong B, Gilchrest H, Anthes JC, Greenfeder S (2005) Identification and characterization of splice variants of the human P2X7 ATP channel. *Biochem Biophys Res Commun* 332:17–27
224. Rassendren F, Buell GN, Virginio C et al (1997) The permeabilizing ATP receptor, P2X7. Cloning and expression of a human cDNA. *J Biol Chem* 272:5482–5486
225. Sluyter R (2017) The P2X7 receptor. *Adv Exp Med Biol* 1051:17–53. https://doi.org/10.1007/5584_2017_59
226. Feng YH, Li X, Wang L et al (2006) A truncated P2X7 receptor variant (P2X7-j) endogenously expressed in cervical cancer cells antagonizes the full-length P2X7 receptor through hetero-oligomerization. *J Biol Chem* 281:17228–17237
227. Jiang LH, Baldwin JM, Roger S, Baldwin SA (2013) Insights into the molecular mechanisms underlying mammalian P2X7 receptor functions and contributions in diseases, revealed by structural modeling and single nucleotide polymorphisms. *Front Pharmacol* 4:55. <https://doi.org/10.3389/fphar.2013.00055>
228. Adinolfi E, Cirillo M, Woltersdorf R et al (2010) Trophic activity of a naturally occurring truncated isoform of the P2X7 receptor. *FASEB J* 24:3393–3404. <https://doi.org/10.1096/fj.09-153601>
229. Gallagher JA, Wiley JS (2012) Polymorphisms in the P2X7 receptor gene are associated with low lumbar spine bone mineral density and accelerated bone loss in postmenopausal women. *Eur J Hum Genet* 20:559–564. <https://doi.org/10.1038/ejhg.2011.245>
230. Xing S, Grol MW, Grutter PH et al (2016) Modeling interactions among individual P2 receptors to explain complex response patterns over a wide range of ATP concentrations. *Front Physiol* 7:294. <https://doi.org/10.3389/fphys.2016.00294>
231. Lenertz LY, Gavala ML, Zhu Y, Bertics PJ (2011) Transcriptional control mechanisms associated with the nucleotide receptor P2X7, a critical regulator of immunologic, osteogenic, and neurologic functions. *Immunol Res* 50:22–38. <https://doi.org/10.1007/s12026-011-8203-4>
232. Di Virgilio F, Schmalzing G, Markwardt F (2018) The Elusive P2X7 Macropore. *Trends Cell Biol* 28:392–404. <https://doi.org/10.1016/j.tcb.2018.01.005>
233. Agrawal A, Gartland A (2015) P2X7 receptors: role in bone cell formation and function. *J Mol Endocrinol* 54:75–88. <https://doi.org/10.1530/JME-14-0226>
234. Gavala ML, Hill LM, Lenertz LY et al (2010) Activation of the transcription factor FosB/activating protein-1 (AP-1) is a prominent downstream signal of the extracellular nucleotide receptor P2RX7 in monocytic and osteoblastic cells. *J Biol Chem* 285(44):34288–34298. <https://doi.org/10.1074/jbc.M110.142091>
235. Wagner EF (2010) Bone development and inflammatory disease is regulated by AP-1 (Fos/Jun). *Ann Rheum Dis* 69(Suppl 1):i86–i88. <https://doi.org/10.1136/ard.2009.119396>
236. Sindhavajiva PR, Sastravaha P, Arskornnukit M, Pavasant P (2017) Purinergic 2X7 receptor activation regulates WNT signaling in human mandibular-derived osteoblasts. *Arch Oral Biol* 81:167–174. <https://doi.org/10.1016/j.archoralbio.2017.05.009>
237. Sun D, Junger WG, Yuan C et al (2013) Shockwaves induce osteogenic differentiation of human mesenchymal stem cells through ATP release and activation of P2X7 receptors. *Stem Cells* 31(6):1170–1180. <https://doi.org/10.1002/stem.1356>
238. Gartland A, Di Virgilio F, Adinolfi E (2014) Trophic activity of human P2X7 receptor isoforms A and B in osteosarcoma. *PLoS ONE* 9:e107224. <https://doi.org/10.1371/journal.pone.0107224>
239. Jiang LH, Mousawi F, Yang X, Roger S (2017) ATP-induced Ca(2+)-signalling mechanisms in the regulation of mesenchymal stem cell migration. *Cell Mol Life Sci* 74:3697–3710. <https://doi.org/10.1007/s00018-017-2545-6>
240. Yu X, Quan J, Long W et al (2018) LL-37 inhibits LPS-induced inflammation and stimulates the osteogenic differentiation of BMSCs via P2X7 receptor and MAPK signaling pathway. *Exp. Cell Res* 372(2):178–187. <https://doi.org/10.1016/j.yexcr.2018.09.024>
241. Li W, Li G, Zhang Y et al (2015) Role of P2X7 receptor in the differentiation of bone marrow stromal cells into osteoblasts and

- adipocytes. *Exp Cell Res* 339(2):367–379. <https://doi.org/10.1016/j.yexcr.2015.10.011>
242. Kariya T, Tanabe N, Shionome C et al (2015) Tension force-induced ATP promotes osteogenesis through P2X7 receptor in osteoblasts. *J Cell Biochem* 116:12–21. <https://doi.org/10.1002/jcb.24863>
243. Jindrichova M, Bhattacharya A, Rupert M et al (2015) Functional characterization of mutants in the transmembrane domains of the rat P2X7 receptor that regulate pore conductivity and agonist sensitivity. *J Neurochem*. 133(6):815–827. <https://doi.org/10.1111/jnc.13078>
244. Jørgensen NR (2018) The purinergic P2X7 ion channel receptor—a ‘repair’ receptor in bone. *Curr Opin Immunol* 52:32–38. <https://doi.org/10.1016/j.coi.2018.03.016>
245. Stoltz JF, de Isla N, Li YP et al (2015) Stem cells and regenerative medicine: myth or reality of the 21st century. *Stem Cells Int*. 2015: 734731. <https://doi.org/10.1155/2015/734731>
246. Eleuteri S, Fierabracci A (2019) Insights into the secretome of mesenchymal stem cells and its potential applications. *Int J Mol Sci* 20(18):pii E4597. <https://doi.org/10.3390/ijms20184597>
247. Kim S, Kim TM (2019) Generation of mesenchymal stem-like cells for producing extracellular vesicles. *World J Stem Cells* 11(5):270–280. <https://doi.org/10.4252/wjsc.v11.i5.270>
248. Praaven LK, Kandoi S, Misra R et al (2019) The mesenchymal stem cell secretome: a new paradigm towards cell-free therapeutic mode in regenerative medicine. *Cytokine Growth Factor Rev* 46: 1–9. <https://doi.org/10.1016/j.cytogfr.2019.04.002>
249. Roura S, Bayes-Genis A (2017) Toward standardization of mesenchymal stromal cell-derived extracellular vesicles for therapeutic use: a call for action. *Proteomics*. 19(1–2):e1800397. <https://doi.org/10.1002/pmic.201800397>
250. Vizoso FJ, Eiro N, Cid S, Schneider J, Perez-Fernandez R (2017) Mesenchymal stem cell secretome: toward cell-free therapeutic strategies in regenerative medicine. *Int J Mol Sci* 18(9):pii: E1852. <https://doi.org/10.3390/ijms18091852>
251. van Balkom BWM, Gremmels H, Giebel B, Lim SK (2019) Proteomic signature of mesenchymal stromal cell-derived small extracellular vesicles. *Proteomics* 19(1–2):e1800163. <https://doi.org/10.1002/pmic.201800163>
252. Qiu G, Zheng G, Ge M et al (2019) Functional proteins of mesenchymal stem cell-derived extracellular vesicles. *Stem Cell Res Ther*. 10(1):359. <https://doi.org/10.1186/s13287-019-1484-6>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.