P2X7 receptors: role in bone cell formation and function

Ankita Agrawal and Alison Gartland

Department of Human Metabolism, The Mellanby Centre for Bone Research, The University of Sheffield, Beech Hill Road, Sheffield S10 2RX, UK

Correspondence should be addressed to A Gartland
Email
a.gartland@sheffield.ac.uk

Abstract

The role of the P2X7 receptor (P2X7R) is being explored with intensive interest in the context of normal bone physiology, bone-related diseases and, to an extent, bone cancer. In this review, we cover the current understanding of P2X7R regulation of bone cell formation, function and survival. We will discuss how the P2X7R drives lineage commitment of undifferentiated bone cell progenitors, the vital role of P2X7R activation in bone mineralisation and its relatively unexplored role in osteocyte function. We also review how P2X7R activation is imperative for osteoclast formation and its role in bone resorption via orchestrating osteoclast apoptosis. Variations in the gene for the P2X7R (*P2RX7*) have implications for P2X7R-mediated processes and we review the relevance of these genetic variations in bone physiology. Finally, we highlight how targeting P2X7R may have therapeutic potential in bone disease and cancer.

Key Words
- apoptosis
- bone
- cancer
- osteoblast
- osteoclast
- P2X7

Journal of Molecular Endocrinology
(2015) **54**, R75–R88

Purinergic signalling

In the last four decades, extensive investigations have led to the recognition of ATP from a 'molecular unit of energy' to an extracellular messenger molecule. ATP-sensitive purinoceptors, omnipresent in vertebrate tissues, are involved in a wide variety of physiological roles (Burnstock 2013) and their function has also been demonstrated in invertebrates (Verkhratsky & Burnstock 2014). While they traditionally act as cell surface sensors (Khakh & North 2006), their participation in signalling within the intracellular compartment in mammals (Qureshi *et al*. 2007, Kuehnel *et al*. 2009, Stokes & Surprenant 2009, Toulme *et al*. 2010) and even protozoans (Fountain *et al*. 2007, Ludlow *et al*. 2009, Sivaramakrishnan & Fountain 2012) has also been recognised.

Purines (adenine, guanine and uridine) can act as signalling molecules in the form of their 5′-nucleotide triphosphates (such as ATP, GTP and UTP), diphosphates (ADP), monophosphate (AMP) or as a nucleoside (adenosine). They can activate one or more of the 19 receptors which are sorted into three classes: P1 (nucleoside) receptors; the metabotropic P2Y receptors and the ionotropic P2X, both of which are nucleotide triggered. Recently, a new family of purine receptors, AdeR or P0-receptors, responsive to adenine has been cloned from rodents (Bender *et al*. 2002, Gorzalka *et al*. 2005, von Kugelgen *et al*. 2008, Thimm *et al*. 2013), although the human homologue is yet to be identified.

Structural and stoichiometrical evidence suggests that all P2X receptor (P2XR) subunits trimerise to form functional receptors (Nicke *et al*. 1998, Barrera *et al*. 2005, Mio *et al*. 2005, Kaczmarek-Hajek *et al*. 2012) with the subunits forming either homomultimers or heteromultimers depending on the subtypes (Burnstock 2007). Each unit comprises two transmembrane domains (TM1 and TM2) with an intervening large extracellular loop and cytoplasmic N- and C-termini, and the primary agonist of all homomeric and heteromeric P2XR is ATP. The current view holds that ligand binding causes reduction in the

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-14-0226

Published by Bioscientifica Ltd.

disulfide bridges between the cystine residues causing the movement of TM1 and TM2, allowing them to open the non-selective cation channel permeable to small monovalent and divalent cations (Browne *et al*. 2010). Subsequent elevation in the intracellular calcium concentration ($[Ca^{2+}]_i$) either by direct Ca^{2+} permeation or by activation of voltage-gated Ca^{2+} channels (Koshimizu *et al*. 2000) triggers a range of signalling cascades, resulting in both short- and long-term cellular events. P2XRs are classed as rapidly desensitising (P2X1 and P2X3) and slowly desensitising (P2X2, P2X4, P2X5 and P2X7) on the basis of the amplitude of the ATP-induced current (Koshimizu *et al*. 2000, North 2002), and continuous agonist application causes an increase in permeability, presumably caused by a progressive rotation and separation of TM1 and TM2 resulting in the formation of a membrane pore (Browne *et al*. 2010).

All the P2XRs share common and specific features as mentioned above, but the P2X7 receptor (P2X7R) differs from the other P2XRs in many ways. First, it has a very long cytoplasmic C-terminal tail, which is responsible for its unique properties and partly mediates P2X7R physiology by interacting with other proteins (Rassendren *et al*. 1997, North 2002, Wilson *et al*. 2002). Secondly, a brief activation of P2X7R results in a rapid membrane depolarisation similar to the other P2XR, but within seconds a more profound development of an additional permeability state occurs (Rassendren *et al*. 1997, Virginio *et al*. 1999). This permeability state allows permeation of larger cations with a molecular weight of up to 900 kDa, such as *N*-methyl-D-glucamine and fluorescence dyes such as the cationic propidium dye YO-PRO-1 and ethidium (Khakh *et al*. 1999), arguably due to an interaction with other proteins such as pannexin hemichannels (Pelegrin & Surprenant 2006). Thirdly, 2,3(4-benzoyl)benzoyl ATP (BzATP) is more potent than ATP at P2X7R whereas ATP is the most potent agonist of other P2XR subtypes and lastly, its activation is well known to induce cellular apoptosis (Zheng *et al*. 1991). Extensive reviews on purinergic signalling in general (Burnstock 2014*a*,*b*,*c*,*d*, Burnstock *et al*. 2014*a*,*b*) and in bone have been recently published (Orriss *et al*. 2010, Reyes *et al*. 2011, Gartland *et al*. 2012*a*, Rumney *et al*. 2012*b*), therefore we focus on P2X7R-mediated signalling in bone in this review.

P2X7R in cells of osteoblast lineage

Progenitor cells

Osteogenic precursors are derived from mesenchymal stem cells (MSCs), and the role of various purinergic receptor in dictating the commitment of MSCs as well as their fate in differentiation has been demonstrated (Zippel *et al*. 2012, Biver *et al*. 2013). Induction of osteogenic differentiation of human MSCs (hMSCs) has recently been shown to occur following shockwave treatment of hMSCs and is thought to be dependent upon P2X7R signalling (Sun *et al*. 2013). The authors demonstrated that cellular ATP was released following shockwave treatment and led to downstream p38 MAPK activation, and to c-Fos and c-Jun mRNA transcription. Treating hMSCs with apyrase (an enzyme that hydrolyses extracellular ATP), P2X7R-siRNA, PPADS (a non-selective P2 antagonist) and KN-62 (a P2X7R antagonist) completely abolished these downstream events, indicating a P2X7R-mediated effect. The shockwave-induced differentiation of MSCs, as measured by alkaline phosphatase activity, osteocalcin production and nodule formation, was significantly reduced by the targeted blockade of P2X7R adding further evidence of a P2X7R-mediated effect (Sun *et al*. 2013). In another study, bone marrow-derived MSC cultures from postmenopausal women showed a P2X7R-dependent enhancement in osteogenic differentiation and mineralisation (Noronha-Matos *et al*. 2014). The study described an initiating event underlying P2X7R-mediated plasma membrane blebbing in hMSC, which was induced by application of BzATP (100 μM) and involved cytoskeleton rearrangements due to P2X7R-dependent protein kinase C (PKC) and Rho-associated kinase activation. In addition, a delayed increase in the basal ALP activity of MSCs from postmenopausal women compared with those from the younger females was also demonstrated, suggestive of an impaired osteogenic commitment in ageing MSCs. However, assessment of ALP activity, expression of the transcription factors RUNX2 and osterix, mineralised area and the number of bone nodules revealed that osteogenic differentiation and mineralisation in postmenopausal MSC cultures could be restored by P2X7R activation with BzATP (Noronha-Matos *et al*. 2014), highlighting the role of P2X7R in promoting mineralisation by MSCs. Taken together, these findings suggest that P2X7R promotes the differentiation of MSCs into mature bone cells and could potentially be used to positively drive bone formation.

Mature cells

As osteoblasts are responsible for bone formation (organic matrix, primarily composed of type I collagen with and eventual deposition of hydroxyapatite mineral), they are essential for the maintenance of bone mass. P2X7R expression has been consistently reported in human

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-14-0226

Published by Bioscientifica Ltd.

and rodent cells of osteoblast lineage (osteoblast-like cell lines, calvarial and bone-derived primary osteoblasts) by RT-PCR, immunochemistry and cell permeabilisation experiments (Nakamura *et al.* 2000, Gartland *et al.* 2001, Orriss *et al.* 2006). It is noteworthy that only a subpopulation of bone-derived and calvarial osteoblasts demonstrate a positive nucleotide response (Gartland *et al.* 2001, Ke *et al.* 2003, Panupinthu *et al.* 2008) indicative of heterogeneity either in the cells in these *in vitro* assays or in P2X7R expression by the cells.

Biological effects of P2X7R activation include apoptosis in SaOS-2 osteoblast cell line (Gartland *et al.* 2001), induction of membrane blebbing in mouse calvarial osteoblasts and MC3T3-E1 osteoblastic cells (Panupinthu *et al.* 2007, Grol *et al.* 2012), production of lipid mediators in mouse calvarial osteoblasts (Panupinthu *et al.* 2008), substantial induction of transcription factor activating protein-1 (AP-1) in mouse MC3T3-E1 cells (Gavala *et al.* 2010) and reduced bone mineralisation and alkaline phosphatase activity in primary rat osteoblasts (Orriss *et al.* 2013). In addition, blockade or absence of P2X7R has been shown to inhibit propagation of intercellular calcium signalling between osteoblasts and osteoclasts in human bone marrow-derived cells (Jorgensen *et al.* 2002), significantly reduce ERK phosphorylation in response to fluid shear stress in mouse primary osteoblasts (Liu *et al.* 2008, Okumura *et al.* 2008) and prevented fluid shear-stress-induced IκBα degradation and nuclear accumulation of nuclear factor kappa B (NFκB) in MC3T3-E1 osteoblasts (Genetos *et al.* 2011).

It is evident that functional P2X7R is required during osteogenesis (Fig. 1); however, contradicting evidence for the effects of P2X7R activation on osteoblast differentiation and matrix mineralisation *in vitro* makes the underlying mechanisms unclear. While rat calvariae-derived osteoblasts show inhibited mineralisation in the presence of P2X7R agonists (Orriss *et al.* 2012), a previous study showed an opposite effect of increased osteoblast differentiation and matrix mineralisation following P2X7R activation in cultures obtained from the same source (Panupinthu *et al.* 2008). The differences between culture methodologies in the two studies could in part explain the discrepancies in these studies. Orriss *et al.* attribute their findings to a P2 receptor-dependent and/or receptor-independent mechanism via hydrolysis of extracellular nucleotides to pyrophosphate (PPi). Indeed, the negative bone mineralisation effect of endogenously released extracellular ATP has subsequently been shown to be averted with apyrase treatment suggestive of an anti-osteogenic autocrine/paracrine mechanism involving other purinergic

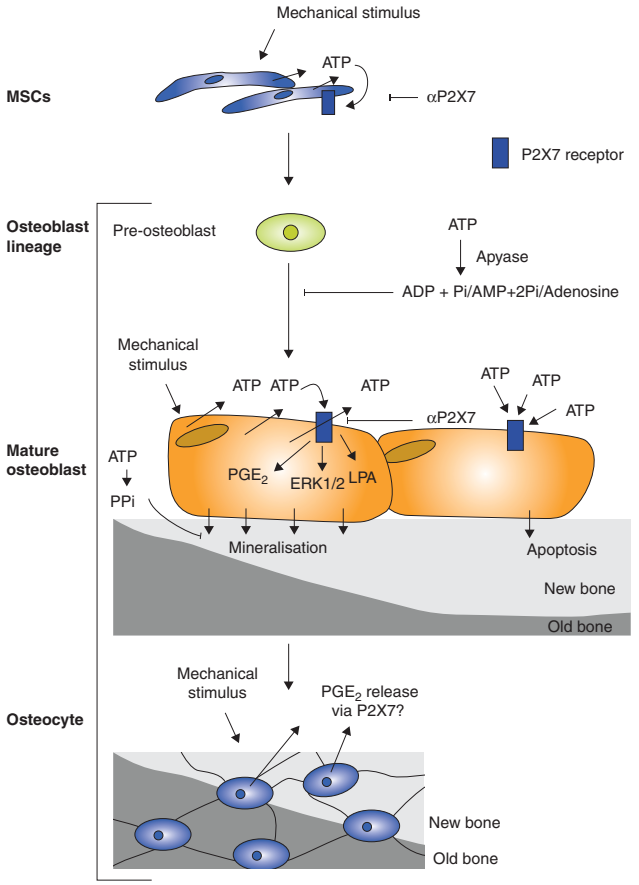

Figure 1
Effect of P2X7R on osteogenesis: osteoblasts are derived from mesenchymal stem cells (MSCs) and cellular ATP release activates P2X7R, driving osteogenic differentiation of MSCs. Hydrolysis of extracellular ATP generates ATP derivatives and pyrophosphate (PPi), both of which contribute negatively to osteoblast function while extracellular ATP (basal or mechanical stimuli induced) has an osteogenic effect. P2X7R signalling leads to downstream events including PGE_2 synthesis/release, LPA synthesis/release and ERK1/2 activation enhancing osteoblast differentiation and bone formation. Mechanical stimulus triggers ATP release, possibly via P2X7R, thereby augmenting receptor mediated osteogenesis. Agonist mediated transient P2X7R activation promotes osteoblast differentiation and matrix mineralisation; however, sustained stimulus is anti-osteogenic caused by accumulation of extracellular ATP evoking apoptosis. During the process of bone formation, some osteoblasts become incorporated within the mineralised matrix as osteocytes. There is evidence of functional P2X7R in these matrix embedded cells, but involvement of P2X7R activation in downstream signalling in osteocytes is unclear.

receptors and ATP derivatives (Orriss *et al.* 2013). In addition, PPi is a known inhibitor of mineralisation and given that biologically relevant PPi levels can be achieved with a single dose of 10 μM ATP or UTP in osteoblast cultures (Orriss *et al.* 2007), PPi generation could be a contributing mechanism to inhibit bone mineralisation. Panupinthu *et al.* (2008) describe that P2X7R-mediated production of lysophosphatidic acid (LPA) metabolites contributes to

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-14-0226

Published by Bioscientifica Ltd.

enhanced osteogenesis observed in their *in vitro* model. LPA signalling involves Rho-associated kinase, which has established roles in driving non-committed cells towards osteoblast lineage (McBeath *et al.* 2004). Therefore, it is likely that the observations of Panupinthu *et al.* involve osteoblast-autonomous mechanisms downstream of P2X7R activation. Further evidence for a positive role of P2X7R in osteoblasts include the observations of reduced ALP activity in osteoblasts from P2X7R knock out (KO) *in vitro* (Panupinthu *et al.* 2008), decreased periosteal bone formation in long bones of P2X7R KO mice (Ke *et al.* 2003) and their reduced osteogenesis in response to mechanical loading (Li *et al.* 2005). Furthermore, the significance of these observations is confirmed in humans as several polymorphisms in the *P2RX7* imparting reduced function to the P2X7R are associated with increased osteoporosis risk in different human cohorts (Ohlendorff *et al.* 2007, Gartland *et al.* 2012*b*, Jorgensen *et al.* 2012). In addition, the truncated P2X7R isoform, P2X7RB, when co-expressed with the full variant P2X7RA demonstrates a significant enhancement of mineralisation in human osteosarcoma cell line (Giuliani *et al.* 2014), thus consolidating a positive role for fully functional P2X7R in maintenance of bone strength.

Other effects of P2X7R activation on osteoblast function include phospholipase D and A_2 stimulation (Panupinthu *et al.* 2007, 2008) and mechanical stress-induced prostaglandin E_2 release (Li *et al.* 2005), suggesting a coupling of P2X7R signalling with production of lipid mediators. In addition, Ca^{2+} influx following P2X7R activation causes sustained proton efflux dependent on glucose and phosphatidylinositol 3-kinase activity (Grol *et al.* 2012) and activation of PKC to mediate phosphorylation of ERK1/2 (Liu *et al.* 2008, Okumura *et al.* 2008), suggestive of more cross talk in osteoblast-like cells. A fluid sheer stress-induced NF-κB nuclear localisation independent of both ERK1/2 LPA signalling has also been demonstrated through P2X7R in osteoblasts (Genetos *et al.* 2011). It seems likely that while the basal/transient activation of P2X7R is osteogenic, sustained stimulation could inhibit the function and activity of these bone-forming cells. In this context, it has been shown that while short-term application of BzATP induces reversible membrane blebbing without activating the key apoptotic mediator caspase-3 in murine osteoblastic cells (Li *et al.* 2005, Panupinthu *et al.* 2007), longer agonist stimulus in human osteoblast-like and primary bone-derived cells caused extensive plasma membrane blebbing and ultimately cell apoptosis (Gartland *et al.* 2001, Alqallaf *et al.* 2009). Table 1 summarises the effect of P2X7R expression on osteoblasts.

P2X7R in osteocytes

Osteocytes are terminally differentiated osteoblast cells which become incorporated within the mineralised matrix in the process of bone formation. Their slender cytoplasmic processes extend and interconnect to communicate with other bone cells on the bone surface, influencing bone remodelling. These cells are difficult to study because they are embedded within the bone, and how these osteocytes relay information to the osteoclasts and osteoblasts and whether purinergic signalling is involved in the process are still not clear.

Despite this, there is support for a role of the P2X7R in osteocyte signalling, with evidence of P2X7R protein expression and BzATP-induced pore formation in MLO-Y4 osteocytes provided nearly 10 years ago (Li *et al.* 2005). Furthermore, P2X7R-induced pore formation in MLO-Y4 osteocytes was shown to occur in response to fluid shear stress and led to the activation of downstream signals typically involved in mechanically induced bone formation, in particular the release of PGE_2 (Yoshida *et al.* 2002, Li *et al.* 2007). However, contradictory evidence suggests that inhibiting P2X7R does not prevent fluid flow-induced release of PGE_2 from MLO-Y4 osteocytes (Cherian *et al.* 2005). While a role of P2X7R activation in regulating mechanical load by osteocytes could be speculated, exactly how the activated ion channel creates an intracellular signal capable of an amplified mechanotransduction process remains unclear. Table 1 summarises the effect of P2X7R expression on osteoclasts.

P2X7R in cells of osteoclast lineage

Progenitor cells

Bone resorbing osteoclasts differentiate from the haematopoietic stem cells (HSCs) and cells of HSC lineage have also been shown to express an array of purinergic receptors (Lemoli *et al.* 2004, Wang *et al.* 2004*a*). A more specific role for the P2X7R has been suggested due to the observations that ATP at high concentrations (1 mM) reduced the number of murine HSCs, whereas the number of more committed, myeloid cells increased (Barbosa *et al.* 2011). The increased proliferation of HSCs caused a reduction in Notch expression, a marker of HSC quiescence, and compromised the ability of HSCs to repopulate the bone marrow. Concentrations lower than 1 mM failed to induce significant changes, indicating a P2X7R-mediated change in murine HSCs to a differentiated state from their primitive, undifferentiated state (Barbosa *et al.* 2011) and therefore suggest a role of P2X7R in promotion of haematopoiesis likely along the osteoclastic lineage.

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-14-0226

Published by Bioscientifica Ltd.

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-14-0226

Published by Bioscientifica Ltd.

Table 1 Effects of P2X7 receptor on bone cells

Lineage	Stage	Species/source	Stimulus	Downstream signalling	Function	References
Osteoblast	Progenitors	Human/MSCs	Shockwave treatment or agonist application	p38 MAPK activation, and c-Fos and c-Jun mRNA transcription PKC and Rho-associated kinase activation	Enhanced osteogenic differentiation and mineralisation	Sun *et al*. (2013) and Noronha-Matos *et al*. (2014)
	Mature	Rat/calvariae	Agonist application	Production of LPA metabolites	Increased osteoblast differentiation and matrix mineralisation	Panupinthu *et al*. (2008), Orriss *et al*. (2012) and Orriss *et al*. (2013)
				Hydrolysis of extracellular nucleotides to pyrophosphate	Reduced bone mineralisation	
		Mouse/primary cells and cell lines	Agonist application	Production of lipid mediators Induction of transcription factor FosB/AP-1 Sustained proton efflux and PKC activation Fluid shear-stress-induced nuclear accumulation of NF-κB Reversible plasma membrane blebbing	Increased osteoblast differentiation and matrix mineralisation	Li *et al*. (2005), Panupinthu *et al*. (2007), Liu *et al*. (2008), Okumura *et al*. (2008), Panupinthu *et al*. (2008), Gavala *et al*. (2010), Genetos *et al*. (2011) and Grol *et al*. (2012)
		Human/primary cells and cell lines	Agonist application	Extensive plasma membrane blebbing	Cell apoptosis	Gartland *et al*. (2001) and Alqallaf *et al*. (2009)
Osteocytes		Mouse/cell line	Fluid shear stress	Contradictory evidence for PGE_2 release	Mechanotransduction	Yoshida *et al*. (2002), Cherian *et al*. (2005) and Li *et al*. (2007)
Osteoclast	Progenitors	Mouse/HSCs	Agonist application	Reduction in Notch expression	Increased differentiation into myeloid cells	Barbosa *et al*. (2011)
	Precursors	Mouse/primary cells and RAW264.7	Knockdown	Prevents NF-κB nuclear localisation, PKC translocation down regulation of NFATc1, cathepsin K, TRAP, AT6v0d2, c-Src, c-Jun and Car2, and suppression of the pro-osteoclastic effect of LPA	Inhibition of cell fusion. Reduction in osteoclast formation and activity	Korcok *et al*. (2004), Armstrong *et al*. (2009) and Hwang *et al*. (2013)
		Human/peripheral blood	Pharmacological blockade	Prevents ATP release via the P2X7R pore	Inhibition of cell fusion	Gartland *et al*. (2003*a*), Agrawal *et al*. (2010) and Pellegatti *et al*. (2011)
	Mature	Mouse/primary cells	Agonist application	Disruption of osteoclastic cytoskeleton via mitochondrial energy regulation	Decreased osteoclast survival and bone resorption	Miyazaki *et al*. (2012)
		Human/peripheral blood and HEK293	Agonist application or P2X7R transfection	Activation of NFATc1	Increase in proliferation in serum-free conditions and resistance to apoptosis in transfectants	Adinolfi *et al*. (2009) and Agrawal & Gartland (2011)
		Human/peripheral blood	Agonist application	Reorganisation of cytoskeleton and secretion of lytic granules at osteoclast-matrix attachment site	Increased bone resorption	Hazama *et al*. (2009)

Mature cells

Osteoclasts, the bone-resorbing cells, are derived from monocytes and the P2X7R has a complex role in these cells of haematopoietic lineage (Fig. 2). P2X7R expression has been shown on authentic osteoclasts generated *in vitro* from small mammals such as rodents and rabbit (Hoebertz *et al*. 2000, Naemsch *et al*. 2001, Orriss *et al*. 2011) and has also confirmed in humans osteoclasts by immunocytochemistry both *in vitro* and *in vivo* (Jorgensen *et al*. 2002, Gartland *et al*. 2003*a*). Using RT-PCR, P2X7R expression was constitutively detected in human monocytic precursors and throughout osteoclastogenesis *in vitro* (Buckley *et al*. 2002, Gartland *et al*. 2003*a*). However, in a recent study, P2X7R expression in primary mouse osteoclasts has been shown to be differentiation dependent, with higher mRNA and protein levels present in mature, resorbing cells compared with their precursors (Brandao-Burch *et al*. 2012). These confounding results in the expression of P2X7R mRNA transcripts could be attributed to the P2X7R specific differences in the investigated species (Roger *et al*. 2010, Bartlett *et al*. 2014), the different source of precursors used to derive mature osteoclasts and a potential contribution of human and murine P2X7R gene variants.

The functional consequence of P2X7R expression has also been extensively investigated. Blockade of P2X7R by a monoclonal antibody against the receptor's external domain or specific P2X7R antagonists prevented osteoclast fusion, but not cell clumping, as previously described by our group (Gartland *et al*. 2003*a*, Agrawal *et al*. 2010). The role of P2X7R in cell fusion is consistent with previous findings in macrophage cell clones, where cells expressing the P2X7R fused spontaneously *in vitro* whereas the ones lacking P2X7R did not (Di Virgilio *et al*. 1999). However, P2X7R KO mice maintain their ability to form multinucleated osteoclasts *in vivo* and *in vitro* (Gartland *et al*. 2003*b*, Ke *et al*. 2003), suggesting that P2X7R might not play an exclusive role in driving cell fusion. Indeed, Pellegatti *et al*. recently demonstrated the existence of an ATP-mediated signalling loop controlling osteoclast fusion. In their study, addition of apyrase, and subsequent accumulation of adenosine, drove fusion whereas pharmacological blockade of P2X7R prevented fusion (Pellegatti *et al*. 2011). Therefore, the authors speculate that ATP release via the P2X7R pore is needed for osteoclastic fusion, although the effect may be indirect involving other purinergic receptors. Since then, the role of P2X7R in driving LPA-stimulated osteoclast fusion has been highlighted (Hwang *et al*. 2013). The authors showed that

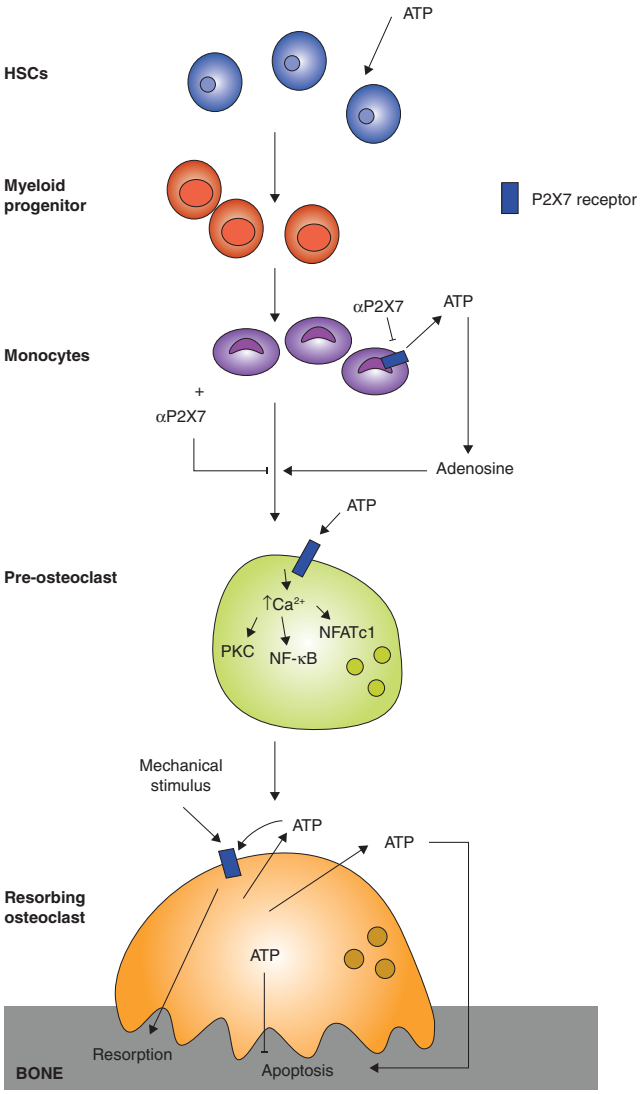

Figure 2
Effect of P2X7R on osteoclastogenesis: extracellular ATP induces commitment of haematopoietic stem cells (HSCs) towards a more committed, myeloid progenitor/pre-osteoclast cell. Fusion of osteoclast precursors is blocked by antagonising P2X7R, possibly as P2X7R pore-mediated ATP release is needed for generation of adenosine which drives fusion. While it is clear that P2X7R activation promotes osteoclastogenes via downstream events such as translocation of PKC, NF-κB nuclear localisation, NFATc1 activation, conflicting evidence exists regarding the significance of extracellular ATP in regulation of bone resorption. High extracellular ATP levels have been shown to both induce resorption and reduce resorption by initiating apoptosis. Intracellular ATP levels positively regulate survival and resorption.

siRNA-induced P2X7R knockdown in RAW 264.7 cells downregulated the expression of osteoclastic functional makers including nuclear factor of activated T cells 1 (NFATc1), cathepsin K, tartrate-resistant acid phosphatase (TRAP), AT6v0d2, c-Src, c-Jun, and Car2, in addition to suppression of the LPA-stimulated increase in osteoclast

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-14-0226

Published by Bioscientifica Ltd.

diameter and bone resorptive capacity in differentiating cultures. The study suggests that P2X7R regulates the positive effect of LPA in osteoclast fusion and possibly also couples to other osteoclastogenic receptors such as osteoclast stimulatory transmembrane protein (OC-STAMP), providing important evidence in support of the role of P2X7R in osteoclastogenesis. It would be interesting to identify whether P2X7R is upstream of OC-STAMP in addition to confirming the findings using mature, resorbing osteoclasts to fully elucidate the effect of P2X7R activation on osteoclast formation.

P2X7R activation is also thought to be important in the differentiation and survival of osteoclasts in both a paracrine and autocrine manner. Stimulus by P2X7R specific agonist induces the membrane depolarisation in osteoclasts (Naemsch *et al*. 2001) and a subsequent increase in intracellular [Ca^{2+}]. This P2X7R-mediated [Ca^{2+}] increase will result in the activation of key signalling molecules, such as PKC, NF-κB, and NFATc1, which are dependent on elevations in cytosolic [Ca^{2+}], and regulate an array of genes involved in osteoclast differentiation. Absence of P2X7R function causes loss of agonist-induced membrane translocation of PKC (Armstrong *et al*. 2009), a signal essential for osteoclast survival (Pereverzev *et al*. 2008); absence of NF-κB nuclear localisation (Korcok *et al*. 2004), a transcription factor essential for osteoclastogenesis (Iotsova *et al*. 1997); and a loss of coupling leading to NFATc1 activation (Ferrari *et al*. 1999, Adinolfi *et al*. 2009), a master regulator of osteoclast differentiation (Takayanagi *et al*. 2002), further highlighting the direct involvement of P2X7R in intracellular signalling crucial to osteoclast differentiation and survival. We have data indicating that nuclear translocation of NFATc1 in RANKL and M-CSF primed monocytes and in mature, resorbing osteoclasts indeed depends on P2X7R activation (Agrawal & Gartland 2011), strengthening the role of P2X7R mediated events during differentiation of these bone resorbing cells. It seems likely that abolishing P2X7R activity would interfere with above signalling activities, thereby negatively influencing osteoclast function by affecting formation of ruffled border and subsequently bone resorption. In this regard, Hazama *et al*. (2009) reported that treatment of human osteoclasts with either BzATP or high concentrations of ATP increased bone resorption *in vitro*. Furthermore, the induction of resorption was accompanied by formation of sealing-zone like structures via the reorganisation of pre-existing cytoskeleton and the secretion of lytic granules at the site of osteoclast–matrix attachment. This seeming P2X7R-mediated augmentation of resorption appears to be absent in the presence of Brilliant Blue G, a selective P2X7R antagonist (Jiang *et al*. 2000), or in osteoclasts from KO mice (Armstrong *et al*. 2009, Hazama *et al*. 2009). Contrary to this, a recent study using murine bone marrow-derived osteoclasts has reported that extracellular ATP caused disruption of murine osteoclastic cytoskeleton and a subsequent reduction in survival and resorption (Miyazaki *et al*. 2012), probably by initiation of apoptosis as suggested previously (Ohlendorff *et al*. 2007). Miyazaki *et al*. show that osteoclastic bone resorption relies on the levels of intracellular ATP, which are dependent on mitochondrial function and steady extracellular ATP levels, as depletion of intracellular ATP led to increased resorption but shorter cell survival. Furthermore, either ATP hydrolysis or repletion of intracellular ATP by expression of anti-apoptotic protein Bcl-$_{xL}$ completely reversed the inhibitory effect of extracellular ATP on osteoclast survival. Considering these two studies together suggests that extracellular ATP can act via two distinct mechanisms in controlling osteoclast cell survival – activation of purinergic signalling, particularly the P2X7R, and the control of mitochondrial energy regulation. However, the contradictory effects of ATP in these two studies remain unexplained, although some differences could be species specific (Donnelly-Roberts *et al*. 2009, Bartlett *et al*. 2014).

Activation of P2X7R in osteoclasts is imperative for cell fusion, can lead to initiation of apoptosis and is critical in determining the duration of cell survival and overall resorption (Table 1). It could be speculated that basal stimulus may cause a hypo or hyper stimulation of P2X7R due to its genetic variations, culminating in enhanced or reduced osteoclast formation and function respectively. Clearly a fine balance between the downstream consequences of P2X7R activation is needed and this may be achieved by modulating extracellular ATP concentrations.

P2X7R-mediated ATP release by bone cells

There are several ways in which ATP can be released from the cell. A non-specific mechanism, following cell trauma, causes cytosolic ATP release along with the rest of the cytoplasmic contents and was probably the initial mechanism from which the entire purinergic network is thought to be evolved (Burnstock & Verkhratsky 2009). Controlled ATP release is thought to occur via vesicular exocytosis, in which ATP is part of a secretory vesicle; transmembrane ATP-binding cassette (ABC) proteins; gap junctions involving connexion and pannexin hemichannels and more recently the P2X7R.

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-14-0226

Published by Bioscientifica Ltd.

ATP release in the context of bone has been shown to vary with the differentiation state of the cell (mature, bone-forming osteoblasts releasing up to several fold more ATP than undifferentiated, proliferating cells) (Orriss *et al*. 2009, Brandao-Burch *et al*. 2012), and the duration, direction and type of mechanical stimulus (Rumney *et al*. 2012*a*). The role of P2X7R regulated ATP release in response to fluid flow was initially described using human osteoblastic cell lines (Rumney *et al*. 2010). Recently, this has been confirmed using rat calvariae-derived osteoblasts (Brandao-Burch *et al*. 2012). However, the finding that P2X7R specific antagonists block ATP release from osteoblast by between 25 and 80% (Brandao-Burch *et al*. 2012) suggests that not all ATP release is via the P2X7R. Moreover, cultured calvarial osteoblasts from P2X7R null mice were previously shown to release similar amounts of ATP compared with WT cells following external stimulus such as fluid shear (Li *et al*. 2005). It is therefore likely that another pathway, such as gap junctions, could be involved. We have shown that ATP release from human osteoclasts derived from peripheral blood monocytes was reduced in the presence of P2X7R specific antagonists (Rumney *et al*. 2011), suggesting involvement of the P2X7R in ATP release from osteoclasts also. In support of this, ATP release through the P2X7R pore has been shown to be an important source of extracellular adenosine which acts to promote fusion of human osteoclast monocyte precursors (Pellegatti *et al*. 2011). In addition, constitutive release of ATP into extracellular microenvironment between 0.05 and 0.5 pmol/ml per cell was inhibited by at least 60% using commercially available P2X7R antagonists during murine osteoclastogenesis (Brandao-Burch *et al*. 2012).

While the exact mechanism of P2X7R-mediated ATP efflux from osteoblasts, osteocytes and osteoclasts remains unclear, it is likely that the participation of the P2X7R in ATP release may be indirect.

P2rx7 KO mice: bone phenotypes

The most extensively used strains of P2X7R KO mice are the Pfizer and GSK lines generated in 2001 and 2005 respectively (Solle *et al*. 2001, Chessell *et al*. 2005). Phenotype analysis of GSK KO females revealed no overall overt skeletal phenotype, whilst detailed bone analysis revealed a thickening of cortical bones but no differences in their trabecular bone volume compared with WT controls (Gartland *et al*. 2003*b*). However, Pfizer KO mice of both genders showed reduced total and cortical bone mineral content (BMC) and decreased femoral periosteal circumference, abnormalities associated with the effects of disuse on the skeleton (Ke *et al*. 2003). Furthermore, the effect of *P2rx7* deletion is more pronounced with age, and histomorphometric analyses showed reduced parameters of bone formation (mineralising surface, bone formation rate) with an increase in parameters of bone resorption (osteoclast number, percent osteoclast surface) in KO mice, supportive of a phenotype with an overall reduced bone mass (Ke *et al*. 2003). Compared with the WT controls, these KO mice are also show an apparent reduced sensitivity to mechanical loading (Li *et al*. 2005) and display impaired fracture healing (Li *et al*. 2009) again, abnormalities associated with the effects of disuse on the skeleton. However, recent evidence has revealed that none of these mice are true global KO as P2X7R splice variants have escaped deletion in both the Pfizer and GSK models due to C-terminal truncated and P2X7R(k) variants respectively (Adriouch *et al*. 2002, Nicke *et al*. 2009, Masin *et al*. 2012). As such, earlier studies describing the phenotype of *P2rx7* KO (Table 2) need to be interpreted with caution.

Syberg *et al*. (2012*b*) performed an extensive analysis of the bone phenotype of ten most common inbred strains of mice. The authors showed that strains carrying the mutated 451L allele (C57BL/6 and DBA/2J), which confers a loss of ATP-induced pore formation (Adriouch *et al*. 2002), had weaker bones and lower levels of the bone resorption marker C-telopeptide collagen in comparison with the strains harbouring the functional P451 allele (BALB/cJ and 129X1/SvJ) (Syberg *et al*. 2012*b*). As a follow-up study, the group showed that the bone phenotype of KO mice was influenced by their genetic background as alterations in bone parameters in the KO strain containing *P451* allele (obtained by backcrossing the GSK *P2rx7* KO mice onto BALB/cJ background) in comparison with the strain containing the 451L allele (original C57BL/6) were more pronounced (Syberg *et al*. 2012*a*). Furthermore, the BALB/cJ KO mice showed reduced serum C-terminal telopeptide (CTX), higher bone mineral density (BMD) and increased bone strength compared with their WT littermates (Syberg *et al*. 2012*a*). Whilst a fully functional, more sensitive P2X7R(k) variant has been demonstrated in certain tissues in the original GSK KO (Nicke *et al*. 2009), osteoclasts obtained from the long bones of BALB/cJ KO mice do not express the P2X7R(k) variant (Hansen *et al*. 2011). We also have data confirming the complete absence of P2X7R function in these bone resorbing cells obtained using the BALB/cJ strain. These studies demonstrate the role of the P2X7R in regulation of bone mass and highlight the importance of genetic background when looking at the functional effects of the P2X7R. For a review of use of these

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-14-0226

Published by Bioscientifica Ltd.

Table 2 Bone phenotype of existing *P2rx7* KO mice models

Model	Sex examined	Phenotype			References
		Bone strength measurements	Histomorphometric analysis	Remarks	
Pfizer KO	Males and females (overall reduced bone mass)	Low BMC in trabecular, cortical, and total bone, smaller bone diameters decreased periosteal and endocortical circumferences in femora	Decreased periosteal bone formation and reduced parameters of bone formation (mineralising surface, bone formation rate) with an increase in parameters of bone resorption (osteoclast number, percent osteoclast surface)	Reduced osteogenesis in response to mechanical loading, impaired fracture repair	Ke *et al.* (2003), Li *et al.* (2005, 2009)
GSK KO	Females (no overall overt skeletal phenotype)	Unchanged BMD, increase in cortical thickness in tibia	No significant difference in cancellous bone volume in tibia, no significant difference in the number of osteoclasts in femora		Gartland *et al.* (2003*b*)
C57Bl/6 KO (451L allele)	Females	Increase in whole-body BMD, increase in bone strength	Significant increase in trabecular thickness in the tibia and vertebrae		Syberg *et al.* (2012*a*)
BALB/cJ KO (P451 allele)	Females	High total BMD, BMC and bone area, increased femoral strength	Reduced serum CTX, ALP and no changes in osteocalcin	C57Bl/6 WT carrying the naturally occurring 451L allele has lower BMD, femoral strength and concentration of bone markers compared with BALB/cJ WT. In addition, lower markers for bone formation and resorption in the C57Bl/6 WT vs BALB/cJ WT	Syberg *et al.* (2012*a*)

mice models in other diseases, please refer to Volonte *et al.* (2012) and Bartlett *et al.* (2014).

P2X7R single nucleotide polymorphisms

The murine *P2rx7* gene is polymorphic with 15 non-synonymous SNPs (http://www.ncbi.nlm.nih.gov/snp, access date 7 August 2014) but only the P451L SNP (change of proline at 451 to leucine) found in the cytoplasmic tail of P2X7R is validated with a reduced channel and pore function (Adriouch *et al.* 2002). In humans, however, there are more than 1700 SNPs (http://www.ncbi.nlm.nih.gov/snp, access date 7 August 2014) in the *P2RX7* of which 146 are non-synonymous and only a small number of these have been functionally investigated. Functional *P2RX7* SNPs have been associated with changes in bone turnover, thereby influencing bone quality. In different population-based cohorts, SNPs known to cause a functional change in P2X7R have been correlated with the change in bone strength in postmenopausal women (Gartland *et al.* 2012*b*, Jorgensen *et al.* 2012, Wesselius *et al.* 2013). The loss-of-function (LOF) SNP p.Arg307Gln is associated with higher bone loss in women in their postmenopausal years (Gartland *et al.* 2012*b*, Jorgensen *et al.* 2012), whilst both men and women with the gain-of-function (GOF) SNPs p.Gln460Arg and p.Ala348Thr are protected against bone loss in addition to a reduced fracture risk (Jorgensen *et al.* 2012, Wesselius *et al.* 2013). The findings reveal that loss of P2X7R function imparts weaker bone strength and enhanced bone loss compared with WT or GOF SNPs, data supported by the investigations from mouse models (Syberg *et al.* 2012*a*). The bone phenotype, influenced by loss of osteoclast apoptosis could potentially contribute to an increased fracture risk (Ohlendorff *et al.* 2007) in people with a LOF SNPs; however, the role of these SNPs on bone cell function remains unaddressed. The GOF p.Ala348Thr may increase susceptibility to inflammatory bone disorders such as rheumatoid arthritis (Al-Shukaili *et al.* 2011), further suggesting the role of *P2RX7* SNPs in detection of 'danger signals' such as development of an inflammatory response. These studies suggest that detection of non-synonymous SNPs within the *P2RX7* gene could prove helpful in identifying people at a greater risk of developing diseases and bone disorders.

P2X7R and bone cancer

Bone tissue provides a fertile setting for cancer cells and is a common metastatic site owing to this microenvironment. Signalling mechanisms involving purines and receptors

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-14-0226

Published by Bioscientifica Ltd.

have been implicated and studied in cancer (Di Virgilio 2012). While the effect of ATP in cancer has long been recognised (Rapaport 1983), it is only recently that the role of P2X7R has been explored. P2X7R expressing HEK cells show increased proliferation, reduced apoptosis and more developed vascular network *in vivo* with strong P2X7R positivity in several human cancers (Adinolfi *et al.* 2012*b*). This is contradictory to previous reports indicating a pro-apoptotic effect of P2X7R in tumour cells (Greig *et al.* 2003, Schafer *et al.* 2003, Wang *et al.* 2004*b*, White *et al.* 2005, Shabbir & Burnstock 2009). Whether these differences are due to the potential preferential expression of P2X7R variants by different tumour cells is currently not known. In addition, tonic, as opposed to sustained P2X7R stimulus, might have a growth-promoting, rather than cytotoxic, effect on tumour growth (Adinolfi *et al.* 2005) by triggering growth promoting intracellular signalling events (Adinolfi *et al.* 2009, Di Virgilio *et al.* 2009). As per the current understanding, high levels of extracellular ATP found in the tumour microenvironment (Pellegatti *et al.* 2008) could involve P2X7R signalling in two different scenarios: i) as a death signal, tumour cells downregulate P2X7R expression and thus avoid apoptosis or ii) as a survival/growth-promoting signal, P2X7R expression causes enhanced invasiveness in primary and secondary sites.

The effect of the full-length P2X7RA and the truncated P2X7RB splice variants on bone tumour cell growth and function has recently been explored. Adinolfi *et al.* (2010) show that growth and matrix invasion were enhanced in cells transfected with P2X7RB and in a follow-up study, the variant showed positive expression in highly dense osteosarcomas, a primary tumour originating in long bones of limbs, *in situ* (Giuliani *et al.* 2014). The cells transfected with P2X7RB had the highest growth rate and increased NFATc1 activation. P2X7RB cells also had decreased mineralisation and decreased RANK-L:OPG ratio (Giuliani *et al.* 2014), highlighting the potential of P2X7R as a therapeutic target in osteosarcoma. In another cancer involving the bone, multiple myeloma, P2X7R activation has been associated with cell death in human RPMI-8226 cell line (Farrell *et al.* 2010); however, the contribution of *P2RX7* variants and polymorphisms was not explored. A study by Paneesha *et al.* (2006) showed that that the 1513 A>C SNP of *P2RX7* had no effect on the clinical prognostic markers and survival in patients with myeloma. Whilst a very recent, more comprehensive analysis identified individuals carrying the variant allele of the 151+1g>t polymorphism or a high number of LOF alleles in the *P2RX7* gene to be at a greater risk of multiple myeloma than individuals not carrying these variant alleles (Vangsted *et al.* 2014). It is likely that the LOF alleles impart reduced P2X7R expression or activity and mitigate P2X7R-mediated apoptosis thus facilitating neoplastic cell growth and myeloma development. A higher activation of P2X7R might be needed in such patients for receptor-mediated cancer prevention, as suggested in other cancers (Gorodeski 2009). Cumulatively, the evidence points to involvement of P2X7R activity in cell proliferation and regulation of bone mass in tumourigenic bone neoplasms. A particular feature of all bone cancers is a debilitating pain, recently the blockade or absence of P2X7R function has been shown to exacerbate bone cancer pain, a separate pain state compared with neuropathic and inflammatory pain (Hansen *et al.* 2011). To understand how P2X7R-mediated events might culminate in bone-related cancer, please see Adinolfi *et al.* (2012*a*). While the above studies point to the role of P2X7R in primary bone cancer and related physiology, further investigations exploring the contribution of P2X7RB variant or its co-expression with P2X7RA and downstream signalling in bone cancer metastases are warranted.

Conclusion and future perspectives

Although both osteoblasts and osteoclasts express P2X7R, its function in their regulation remains complex. It is likely that while the basal/transient activation of P2X7R is osteogenic, sustained stimulation inhibits new bone formation and mineralisation. In a similar way, osteoclast formation requires P2X7R activation, but the bone resorptive ability could be inhibited in the presence of a sustained ATP stimulus. The duality of P2X7R signalling in these bone cells is further complicated by the existence of variations in the receptor, caused by splice isoforms and SNPs in the gene for P2X7R. Investigations have shed light on the influence of *P2RX7* gene variations on bone cell function and bone remodelling, and need to be considered in future studies involving human subjects and rodent models. These variants could contribute to a diversity in P2X7R-mediated osteoblast, osteoclast and osteocyte function and overall bone health; therefore, a better understanding of P2X7R-mediated downstream signalling in bone cells would be helpful, particularly in bone-related conditions. Pharmacological blockade of P2X7R shows promising therapeutic option; however, the apparent differential activity between species and also between individuals (Bartlett *et al.* 2014) needs to be carefully considered, and studies with new generation antagonists will be important for supporting a role for P2X7R in bone diseases.

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-14-0226

Published by Bioscientifica Ltd.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding
This work was supported by Arthritis Research UK (grant number 18753); the Breast Cancer Campaign (grant number 2012MayPR086); and European Commission under the 7th Framework Programme (proposal 202231) performed as a collaborative project among the members of the ATPBone Consortium (Copenhagen University, Copenhagen, Denmark; University College London, London, UK; University of Maastricht, Maastricht, The Netherlands; University of Ferrara, Ferrara, Italy; University of Liverpool, Liverpool, UK; University of Sheffield, Sheffield, UK; and Université Libre de Bruxelles, Brussels, Belgium) and is a substudy under the main study, 'Fighting osteoporosis by blocking nucleotides: purinergic signalling in bone formation and homeostasis.'

References

Adinolfi E, Callegari MG, Ferrari D, Bolognesi C, Minelli M, Wieckowski MR, Pinton P, Rizzuto R & Di Virgilio F 2005 Basal activation of the P2X7 ATP receptor elevates mitochondrial calcium and potential, increases cellular ATP levels, and promotes serum-independent growth. *Molecular Biology of the Cell* **16** 3260–3272. (doi:10.1091/mbc.E04-11-1025)

Adinolfi E, Callegari MG, Cirillo M, Pinton P, Giorgi C, Cavagna D, Rizzuto R & Di Virgilio F 2009 Expression of the P2X7 receptor increases the Ca^{2+} content of the endoplasmic reticulum, activates NFATc1, and protects from apoptosis. *Journal of Biological Chemistry* **284** 10120–10128. (doi:10.1074/jbc.M805805200)

Adinolfi E, Cirillo M, Woltersdorf R, Falzoni S, Chiozzi P, Pellegatti P, Callegari MG, Sandona D, Markwardt F, Schmalzing G *et al.* 2010 Trophic activity of a naturally occurring truncated isoform of the P2X7 receptor. *FASEB Journal* **24** 3393–3404. (doi:10.1096/fj.09-153601)

Adinolfi E, Amoroso F & Giuliani AL 2012*a* P2X7 receptor function in bone-related cancer. *Journal of Osteoporosis* **2012** 637863. (doi:10.1155/2012/637863)

Adinolfi E, Raffaghello L, Giuliani AL, Cavazzini L, Capece M, Chiozzi P, Bianchi G, Kroemer G, Pistoia V & Di Virgilio F 2012*b* Expression of P2X7 receptor increases *in vivo* tumor growth. *Cancer Research* **72** 2957–2969. (doi:10.1158/0008-5472.CAN-11-1947)

Adriouch S, Dox C, Welge V, Seman M, Koch-Nolte F & Haag F 2002 Cutting edge: a natural P451L mutation in the cytoplasmic domain impairs the function of the mouse P2X7 receptor. *Journal of Immunology* **169** 4108–4112. (doi:10.4049/jimmunol.169.8.4108)

Agrawal A & Gartland A 2011 Expression of NFATc1 is inducible by P2X7 receptor activation in human osteoclasts. In *European Calcified Tissue Society, International Bone and Mineral Society, Poster Presentation*, p S126. Athens: Bone.

Agrawal A, Buckley KA, Bowers K, Furber M, Gallagher JA & Gartland A 2010 The effects of P2X7 receptor antagonists on the formation and function of human osteoclasts *in vitro*. *Purinergic Signalling* **6** 307–315. (doi:10.1007/s11302-010-9181-z)

Alqallaf SM, Evans BA & Kidd EJ 2009 Atypical P2X receptor pharmacology in two human osteoblast-like cell lines. *British Journal of Pharmacology* **156** 1124–1135. (doi:10.1111/j.1476-5381.2009.00119.x)

Al-Shukaili A, Al-Kaabi J, Hassan B, Al-Araimi T, Al-Tobi M, Al-Kindi M, Al-Maniri A, Al-Gheilani A & Al-Ansari A 2011 P2X7 receptor gene polymorphism analysis in rheumatoid arthritis. *International Journal of Immunogenetics* **38** 389–396. (doi:10.1111/j.1744-313X.2011.01019.x)

Armstrong S, Pereverzev A, Dixon SJ & Sims SM 2009 Activation of P2X7 receptors causes isoform-specific translocation of protein kinase C in osteoclasts. *Journal of Cell Science* **122** 136–144. (doi:10.1242/jcs.031534)

Barbosa CM, Leon CM, Nogueira-Pedro A, Wasinsk F, Araujo RC, Miranda A, Ferreira AT & Paredes-Gamero EJ 2011 Differentiation of hematopoietic stem cell and myeloid populations by ATP is modulated by cytokines. *Cell Death & Disease* **2** e165. (doi:10.1038/cddis.2011.49)

Barrera NP, Ormond SJ, Henderson RM, Murrell-Lagnado RD & Edwardson JM 2005 Atomic force microscopy imaging demonstrates that P2X2 receptors are trimers but that P2X6 receptor subunits do not oligomerize. *Journal of Biological Chemistry* **280** 10759–10765. (doi:10.1074/jbc.M412265200)

Bartlett R, Stokes L & Sluyter R 2014 The P2X7 receptor channel: recent developments and the use of P2X7 antagonists in models of disease. *Pharmacological Reviews* **66** 638–675. (doi:10.1124/pr.113.008003)

Bender E, Buist A, Jurzak M, Langlois X, Baggerman G, Verhasselt P, Ercken M, Guo HQ, Wintmolders C, Van den Wyngaert I *et al.* 2002 Characterization of an orphan G protein-coupled receptor localized in the dorsal root ganglia reveals adenine as a signaling molecule. *PNAS* **99** 8573–8578. (doi:10.1073/pnas.122016499)

Biver G, Wang N, Gartland A, Orriss I, Arnett TR, Boeynaems JM & Robaye B 2013 Role of the P2Y13 receptor in the differentiation of bone marrow stromal cells into osteoblasts and adipocytes. *Stem Cells* **31** 2747–2758. (doi:10.1002/stem.1411)

Brandao-Burch A, Key ML, Patel JJ, Arnett TR & Orriss IR 2012 The P2X7 receptor is an important regulator of extracellular ATP levels. *Frontiers in Endocrinology* **3** 41. (doi:10.3389/fendo.2012.00041)

Browne LE, Jiang LH & North RA 2010 New structure enlivens interest in P2X receptors. *Trends in Pharmacological Sciences* **31** 229–237. (doi:10.1016/j.tips.2010.02.004)

Buckley KA, Hipskind RA, Gartland A, Bowler WB & Gallagher JA 2002 Adenosine triphosphate stimulates human osteoclast activity via upregulation of osteoblast-expressed receptor activator of nuclear factor-κB ligand. *Bone* **31** 582–590. (doi:10.1016/S8756-3282(02)00877-3)

Burnstock G 2007 Purine and pyrimidine receptors. *Cellular and Molecular Life Sciences* **64** 1471–1483. (doi:10.1007/s00018-007-6497-0)

Burnstock G 2013 Introduction and perspective, historical note. *Frontiers in Cellular Neuroscience* **7** 227. (doi:10.3389/fncel.2013.00227)

Burnstock G 2014*a* Purinergic signalling in the gastrointestinal tract and related organs in health and disease. *Purinergic Signalling* **10** 3–50. (doi:10.1007/s11302-013-9397-9)

Burnstock G 2014*b* Purinergic signalling in the urinary tract in health and disease. *Purinergic Signalling* **10** 103–155. (doi:10.1007/s11302-013-9395-y)

Burnstock G 2014*c* Purinergic signalling in the reproductive system in health and disease. *Purinergic Signalling* **10** 157–187. (doi:10.1007/s11302-013-9399-7)

Burnstock G 2014*d* Purinergic signalling in endocrine organs. *Purinergic Signalling* **10** 189–231. (doi:10.1007/s11302-013-9396-x)

Burnstock G & Verkhratsky A 2009 Evolutionary origins of the purinergic signalling system. *Acta Physiologica* **195** 415–447. (doi:10.1111/j.1748-1716.2009.01957.x)

Burnstock G, Evans LC & Bailey MA 2014*a* Purinergic signalling in the kidney in health and disease. *Purinergic Signalling* **10** 71–101. (doi:10.1007/s11302-013-9400-5)

Burnstock G, Vaughn B & Robson SC 2014*b* Purinergic signalling in the liver in health and disease. *Purinergic Signalling* **10** 51–70. (doi:10.1007/s11302-013-9398-8)

Cherian PP, Siller-Jackson AJ, Gu S, Wang X, Bonewald LF, Sprague E & Jiang JX 2005 Mechanical strain opens connexin 43 hemichannels in osteocytes: a novel mechanism for the release of prostaglandin. *Molecular Biology of the Cell* **16** 3100–3106. (doi:10.1091/mbc.E04-10-0912)

Chessell IP, Hatcher JP, Bountra C, Michel AD, Hughes JP, Green P, Egerton J, Murfin M, Richardson J, Peck WL *et al.* 2005 Disruption of the P2X7

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-14-0226

Published by Bioscientifica Ltd.

purinoceptor gene abolishes chronic inflammatory and neuropathic pain. *Pain* **114** 386–396. (doi:10.1016/j.pain.2005.01.002)

Di Virgilio F 2012 Purines, purinergic receptors, and cancer. *Cancer Research* **72** 5441–5447. (doi:10.1158/0008-5472.CAN-12-1600)

Di Virgilio F, Falzoni S, Chiozzi P, Sanz JM, Ferrari D & Buell GN 1999 ATP receptors and giant cell formation. *Journal of Leukocyte Biology* **66** 723–726.

Di Virgilio F, Ferrari D & Adinolfi E 2009 P2X(7): a growth-promoting receptor-implications for cancer. *Purinergic Signalling* **5** 251–256. (doi:10.1007/s11302-009-9145-3)

Donnelly-Roberts DL, Namovic MT, Han P & Jarvis MF 2009 Mammalian P2X7 receptor pharmacology: comparison of recombinant mouse, rat and human P2X7 receptors. *British Journal of Pharmacology* **157** 1203–1214. (doi:10.1111/j.1476-5381.2009.00233.x)

Farrell AW, Gadeock S, Pupovac A, Wang B, Jalilian I, Ranson M & Sluyter R 2010 P2X7 receptor activation induces cell death and CD23 shedding in human RPMI 8226 multiple myeloma cells. *Biochimica et Biophysica Acta* **1800** 1173–1182. (doi:10.1016/j.bbagen.2010.07.001)

Ferrari D, Stroh C & Schulze-Osthoff K 1999 P2X7/P2Z purinoreceptor-mediated activation of transcription factor NFAT in microglial cells. *Journal of Biological Chemistry* **274** 13205–13210. (doi:10.1074/jbc.274.19.13205)

Fountain SJ, Parkinson K, Young MT, Cao L, Thompson CR & North RA 2007 An intracellular P2X receptor required for osmoregulation in *Dictyostelium discoideum*. *Nature* **448** 200–203. (doi:10.1038/nature05926)

Gartland A, Hipskind RA, Gallagher JA & Bowler WB 2001 Expression of a P2X7 receptor by a subpopulation of human osteoblasts. *Journal of Bone and Mineral Research* **16** 846–856. (doi:10.1359/jbmr.2001.16.5.846)

Gartland A, Buckley KA, Bowler WB & Gallagher JA 2003*a* Blockade of the pore-forming P2X7 receptor inhibits formation of multinucleated human osteoclasts *in vitro*. *Calcified Tissue International* **73** 361–369. (doi:10.1007/s00223-002-2098-y)

Gartland A, Buckley KA, Hipskind RA, Perry MJ, Tobias JH, Buell G, Chessell I, Bowler WB & Gallagher JA 2003*b* Multinucleated osteoclast formation *in vivo* and *in vitro* by P2X7 receptor-deficient mice. *Critical Reviews in Eukaryotic Gene Expression* **13** 243–253. (doi:10.1615/CritRevEukaryotGeneExpr.v13.i24.150)

Gartland A, Orriss IR, Rumney RM, Bond AP, Arnett T & Gallagher JA 2012*a* Purinergic signalling in osteoblasts. *Frontiers in Bioscience* **17** 16–29. (doi:10.2741/3912)

Gartland A, Skarratt KK, Hocking LJ, Parsons C, Stokes L, Jorgensen NR, Fraser WD, Reid DM, Gallagher JA & Wiley JS 2012*b* Polymorphisms in the P2X7 receptor gene are associated with low lumbar spine bone mineral density and accelerated bone loss in post-menopausal women. *European Journal of Human Genetics* **20** 559–564. (doi:10.1038/ejhg.2011.245)

Gavala ML, Hill LM, Lenertz LY, Karta MR & Bertics PJ 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. *Journal of Biological Chemistry* **285** 34288–34298. (doi:10.1074/jbc.M110.142091)

Genetos DC, Karin NJ, Geist DJ, Donahue HJ & Duncan RL 2011 Purinergic signaling is required for fluid shear stress-induced NF-κB translocation in osteoblasts. *Experimental Cell Research* **317** 737–744. (doi:10.1016/j.yexcr.2011.01.007)

Giuliani AL, Colognesi D, Ricco T, Roncato C, Capece M, Amoroso F, Wang QG, De Marchi E, Gartland A, Di Virgilio F *et al.* 2014 Trophic activity of human P2X7 receptor isoforms A and B in osteosarcoma. *PLoS ONE* **9** e107224. (doi:10.1371/journal.pone.0107224)

Gorodeski GI 2009 P2X7-mediated chemoprevention of epithelial cancers. *Expert Opinion on Therapeutic Targets* **13** 1313–1332. (doi:10.1517/14728220903277249)

Gorzalka S, Vittori S, Volpini R, Cristalli G, von Kugelgen I & Muller CE 2005 Evidence for the functional expression and pharmacological characterization of adenine receptors in native cells and tissues. *Molecular Pharmacology* **67** 955–964. (doi:10.1124/mol.104.006601)

Greig AV, Linge C, Healy V, Lim P, Clayton E, Rustin MH, McGrouther DA & Burnstock G 2003 Expression of purinergic receptors in non-melanoma skin cancers and their functional roles in A431 cells. *Journal of Investigative Dermatology* **121** 315–327. (doi:10.1046/j.1523-1747.2003.12379.x)

Grol MW, Zelner I & Dixon SJ 2012 P2X(7)-mediated calcium influx triggers a sustained, PI3K-dependent increase in metabolic acid production by osteoblast-like cells. *American Journal of Physiology. Endocrinology and Metabolism* **302** E561–E575. (doi:10.1152/ajpendo.00209.2011)

Hansen RR, Nielsen CK, Nasser A, Thomsen SI, Eghorn LF, Pham Y, Schulenburg C, Syberg S, Ding M, Stojilkovic SS *et al.* 2011 P2X7 receptor-deficient mice are susceptible to bone cancer pain. *Pain* **152** 1766–1776. (doi:10.1016/j.pain.2011.03.024)

Hazama R, Qu X, Yokoyama K, Tanaka C, Kinoshita E, He J, Takahashi S, Tohyama K, Yamamura H & Tohyama Y 2009 ATP-induced osteoclast function: the formation of sealing-zone like structure and the secretion of lytic granules via microtubule-deacetylation under the control of Syk. *Genes to Cells* **14** 871–884. (doi:10.1111/j.1365-2443.2009.01317.x)

Hoebertz A, Townsend-Nicholson A, Glass R, Burnstock G & Arnett TR 2000 Expression of P2 receptors in bone and cultured bone cells. *Bone* **27** 503–510. (doi:10.1016/S8756-3282(00)00351-3)

Hwang YS, Ma GT, Park KK & Chung WY 2013 Lysophosphatidic acid stimulates osteoclast fusion through OC-STAMP and P2X7 receptor signaling. *Journal of Bone and Mineral Metabolism* **32** 110–122. (doi:10.1007/s00774-013-0470-9)

Iotsova V, Caamano J, Loy J, Yang Y, Lewin A & Bravo R 1997 Osteopetrosis in mice lacking NF-κB1 and NF-κB2. *Nature Medicine* **3** 1285–1289. (doi:10.1038/nm1197-1285)

Jiang LH, Mackenzie AB, North RA & Surprenant A 2000 Brilliant blue G selectively blocks ATP-gated rat P2X(7) receptors. *Molecular Pharmacology* **58** 82–88. (doi:1124/mol.58.1.82)

Jorgensen NR, Henriksen Z, Sorensen OH, Eriksen EF, Civitelli R & Steinberg TH 2002 Intercellular calcium signaling occurs between human osteoblasts and osteoclasts and requires activation of osteoclast P2X7 receptors. *Journal of Biological Chemistry* **277** 7574–7580. (doi:10.1074/jbc.M104608200)

Jorgensen NR, Husted LB, Skarratt KK, Stokes L, Tofteng CL, Kvist T, Jensen JE, Eiken P, Brixen K, Fuller S *et al.* 2012 Single-nucleotide polymorphisms in the P2X7 receptor gene are associated with post-menopausal bone loss and vertebral fractures. *European Journal of Human Genetics* **20** 675–681. (doi:10.1038/ejhg.2011.253)

Kaczmarek-Hajek K, Lorinczi E, Hausmann R & Nicke A 2012 Molecular and functional properties of P2X receptors – recent progress and persisting challenges. *Purinergic Signalling* **8** 375–417. (doi:10.1007/s11302-012-9314-7)

Ke HZ, Qi H, Weidema AF, Zhang Q, Panupinthu N, Crawford DT, Grasser WA, Paralkar VM, Li M, Audoly LP *et al.* 2003 Deletion of the P2X7 nucleotide receptor reveals its regulatory roles in bone formation and resorption. *Molecular Endocrinology* **17** 1356–1367. (doi:10.1210/me.2003-0021)

Khakh BS & North RA 2006 P2X receptors as cell-surface ATP sensors in health and disease. *Nature* **442** 527–532. (doi:10.1038/nature04886)

Khakh BS, Bao XR, Labarca C & Lester HA 1999 Neuronal P2X transmitter-gated cation channels change their ion selectivity in seconds. *Nature Neuroscience* **2** 322–330. (doi:10.1038/7233)

Korcok J, Raimundo LN, Ke HZ, Sims SM & Dixon SJ 2004 Extracellular nucleotides act through P2X7 receptors to activate NF-κB in osteoclasts. *Journal of Bone and Mineral Research* **19** 642–651. (doi:10.1359/JBMR.040108)

Koshimizu TA, Van Goor F, Tomic M, Wong AO, Tanoue A, Tsujimoto G & Stojilkovic SS 2000 Characterization of calcium signaling by purinergic receptor-channels expressed in excitable cells. *Molecular Pharmacology* **58** 936–945. (doi:10.1124/mol.58.5.936)

Kuehnel MP, Rybin V, Anand PK, Anes E & Griffiths G 2009 Lipids regulate P2X7-receptor-dependent actin assembly by phagosomes via ADP

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-14-0226

Published by Bioscientifica Ltd.

translocation and ATP synthesis in the phagosome lumen. *Journal of Cell Science* **122** 499–504. (doi:10.1242/jcs.034199)

von Kugelgen I, Schiedel AC, Hoffmann K, Alsdorf BB, Abdelrahman A & Muller CE 2008 Cloning and functional expression of a novel Gi protein-coupled receptor for adenine from mouse brain. *Molecular Pharmacology* **73** 469–477. (doi:10.1124/mol.107.037069)

Lemoli RM, Ferrari D, Fogli M, Rossi L, Pizzirani C, Forchap S, Chiozzi P, Vaselli D, Bertolini F, Foutz T *et al.* 2004 Extracellular nucleotides are potent stimulators of human hematopoietic stem cells *in vitro* and *in vivo*. *Blood* **104** 1662–1670. (doi:10.1182/blood-2004-03-0834)

Li J, Liu D, Ke HZ, Duncan RL & Turner CH 2005 The P2X7 nucleotide receptor mediates skeletal mechanotransduction. *Journal of Biological Chemistry* **280** 42952–42959. (doi:10.1074/jbc.M506415200)

Li M, Thompson DD & Paralkar VM 2007 Prostaglandin E(2) receptors in bone formation. *International Orthopaedics* **31** 767–772. (doi:10.1007/s00264-007-0406-x)

Li J, Meyer R, Duncan RL & Turner CH 2009 P2X7 nucleotide receptor plays an important role in callus remodeling during fracture repair. *Calcified Tissue International* **84** 405–412. (doi:10.1007/s00223-009-9237-7)

Liu D, Genetos DC, Shao Y, Geist DJ, Li J, Ke HZ, Turner CH & Duncan RL 2008 Activation of extracellular-signal regulated kinase (ERK1/2) by fluid shear is Ca(2+)- and ATP-dependent in MC3T3-E1 osteoblasts. *Bone* **42** 644–652. (doi:10.1016/j.bone.2007.09.058)

Ludlow MJ, Durai L & Ennion SJ 2009 Functional characterization of intracellular *Dictyostelium discoideum* P2X receptors. *Journal of Biological Chemistry* **284** 35227–35239. (doi:10.1074/jbc.M109.045674)

Masin M, Young C, Lim K, Barnes SJ, Xu XJ, Marschall V, Brutkowski W, Mooney ER, Gorecki DC & Murrell-Lagnado R 2012 Expression, assembly and function of novel C-terminal truncated variants of the mouse P2X7 receptor: re-evaluation of P2X7 knockouts. *British Journal of Pharmacology* **165** 978–993. (doi:10.1111/j.1476-5381.2011.01624.x)

McBeath R, Pirone DM, Nelson CM, Bhadriraju K & Chen CS 2004 Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Developmental Cell* **6** 483–495. (doi:10.1016/S1534-5807(04)00075-9)

Mio K, Kubo Y, Ogura T, Yamamoto T & Sato C 2005 Visualization of the trimeric P2X2 receptor with a crown-capped extracellular domain. *Biochemical and Biophysical Research Communications* **337** 998–1005. (doi:10.1016/j.bbrc.2005.09.141)

Miyazaki T, Iwasawa M, Nakashima T, Mori S, Shigemoto K, Nakamura H, Katagiri H, Takayanagi H & Tanaka S 2012 Intracellular and extracellular ATP coordinately regulate the inverse correlation between osteoclast survival and bone resorption. *Journal of Biological Chemistry* **287** 37808–37823. (doi:10.1074/jbc.M112.385369)

Naemsch LN, Dixon SJ & Sims SM 2001 Activity-dependent development of P2X7 current and Ca^{2+} entry in rabbit osteoclasts. *Journal of Biological Chemistry* **276** 39107–39114. (doi:10.1074/jbc.M105881200)

Nakamura E, Uezono Y, Narusawa K, Shibuya I, Oishi Y, Tanaka M, Yanagihara N, Nakamura T & Izumi F 2000 ATP activates DNA synthesis by acting on P2X receptors in human osteoblast-like MG-63 cells. *American Journal of Physiology. Cell Physiology* **279** C510–C519.

Nicke A, Baumert HG, Rettinger J, Eichele A, Lambrecht G, Mutschler E & Schmalzing G 1998 P2X1 and P2X3 receptors form stable trimers: a novel structural motif of ligand-gated ion channels. *EMBO Journal* **17** 3016–3028. (doi:10.1093/emboj/17.11.3016)

Nicke A, Kuan YH, Masin M, Rettinger J, Marquez-Klaka B, Bender O, Gorecki DC, Murrell-Lagnado RD & Soto F 2009 A functional P2X7 splice variant with an alternative transmembrane domain 1 escapes gene inactivation in P2X7 knock-out mice. *Journal of Biological Chemistry* **284** 25813–25822. (doi:10.1074/jbc.M109.033134)

Noronha-Matos JB, Coimbra J, Sa ESA, Rocha R, Marinhas J, Freitas R, Guerra-Gomes S, Ferreirinha F, Costa MA & Correia-de-Sa P 2014 P2X7-induced zeiosis promotes osteogenic differentiation and mineralization of postmenopausal bone marrow-derived mesenchymal stem cells. *FASEB Journal* **28** 5208–5222. (doi:10.1096/fj.14-257923)

North RA 2002 Molecular physiology of P2X receptors. *Physiological Reviews* **82** 1013–1067. (doi:10.1152/physrev.00015.2002)

Ohlendorff SD, Tofteng CL, Jensen JE, Petersen S, Civitelli R, Fenger M, Abrahamsen B, Hermann AP, Eiken P & Jorgensen NR 2007 Single nucleotide polymorphisms in the P2X7 gene are associated to fracture risk and to effect of estrogen treatment. *Pharmacogenetics and Genomics* **17** 555–567. (doi:10.1097/FPC.0b013e3280951625)

Okumura H, Shiba D, Kubo T & Yokoyama T 2008 P2X7 receptor as sensitive flow sensor for ERK activation in osteoblasts. *Biochemical and Biophysical Research Communications* **372** 486–490. (doi:10.1016/j.bbrc.2008.05.066)

Orriss IR, Knight GE, Ranasinghe S, Burnstock G & Arnett TR 2006 Osteoblast responses to nucleotides increase during differentiation. *Bone* **39** 300–309. (doi:10.1016/j.bone.2006.02.063)

Orriss IR, Utting JC, Brandao-Burch A, Colston K, Grubb BR, Burnstock G & Arnett TR 2007 Extracellular nucleotides block bone mineralization *in vitro*: evidence for dual inhibitory mechanisms involving both P2Y2 receptors and pyrophosphate. *Endocrinology* **148** 4208–4216. (doi:10.1210/en.2007-0066)

Orriss IR, Knight GE, Utting JC, Taylor SE, Burnstock G & Arnett TR 2009 Hypoxia stimulates vesicular ATP release from rat osteoblasts. *Journal of Cellular Physiology* **220** 155–162. (doi:10.1002/jcp.21745)

Orriss IR, Burnstock G & Arnett TR 2010 Purinergic signalling and bone remodelling. *Current Opinion in Pharmacology* **10** 322–330. (doi:10.1016/j.coph.2010.01.003)

Orriss IR, Wang N, Burnstock G, Arnett TR, Gartland A, Robaye B & Boeynaems JM 2011 The P2Y(6) receptor stimulates bone resorption by osteoclasts. *Endocrinology* **152** 3706–3716. (doi:10.1210/en.2011-1073)

Orriss IR, Key ML, Brandao-Burch A, Patel JJ, Burnstock G & Arnett TR 2012 The regulation of osteoblast function and bone mineralization by extracellular nucleotides: the role of p2x receptors. *Bone* **51** 389–400. (doi:10.1016/j.bone.2012.06.013)

Orriss IR, Key ML, Hajjawi MO & Arnett TR 2013 Extracellular ATP released by osteoblasts is a key local inhibitor of bone mineralization. *PLoS ONE* **8** e69057. (doi:10.1371/journal.pone.0069057)

Paneesha S, Starczynski J, Pepper C, Delgado J, Hooper L, Fegan C & Pratt G 2006 The P2X7 receptor gene polymorphism 1513 A→C has no effect on clinical prognostic markers and survival in multiple myeloma. *Leukemia & Lymphoma* **47** 281–284. (doi:10.1080/10428190500305901)

Panupinthu N, Zhao L, Possmayer F, Ke HZ, Sims SM & Dixon SJ 2007 P2X7 nucleotide receptors mediate blebbing in osteoblasts through a pathway involving lysophosphatidic acid. *Journal of Biological Chemistry* **282** 3403–3412. (doi:10.1074/jbc.M605620200)

Panupinthu N, Rogers JT, Zhao L, Solano-Flores LP, Possmayer F, Sims SM & Dixon SJ 2008 P2X7 receptors on osteoblasts couple to production of lysophosphatidic acid: a signaling axis promoting osteogenesis. *Journal of Cell Biology* **181** 859–871. (doi:10.1083/jcb.200708037)

Pelegrin P & Surprenant A 2006 Pannexin-1 mediates large pore formation and interleukin-1β release by the ATP-gated P2X7 receptor. *EMBO Journal* **25** 5071–5082. (doi:10.1038/sj.emboj.7601378)

Pellegatti P, Raffaghello L, Bianchi G, Piccardi F, Pistoia V & Di Virgilio F 2008 Increased level of extracellular ATP at tumor sites: *in vivo* imaging with plasma membrane luciferase. *PLoS ONE* **3** e2599. (doi:10.1371/journal.pone.0002599)

Pellegatti P, Falzoni S, Donvito G, Lemaire I & Di Virgilio F 2011 P2X7 receptor drives osteoclast fusion by increasing the extracellular adenosine concentration. *FASEB Journal* **25** 1264–1274. (doi:10.1096/fj.10-169854)

Pereverzev A, Komarova SV, Korcok J, Armstrong S, Tremblay GB, Dixon SJ & Sims SM 2008 Extracellular acidification enhances osteoclast survival through an NFAT-independent, protein kinase C-dependent pathway. *Bone* **42** 150–161. (doi:10.1016/j.bone.2007.08.044)

Qureshi OS, Paramasivam A, Yu JC & Murrell-Lagnado RD 2007 Regulation of P2X4 receptors by lysosomal targeting, glycan protection and exocytosis. *Journal of Cell Science* **120** 3838–3849. (doi:10.1242/jcs.010348)

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-14-0226

Published by Bioscientifica Ltd.

Rapaport E 1983 Treatment of human tumor cells with ADP or ATP yields arrest of growth in the S phase of the cell cycle. *Journal of Cellular Physiology* **114** 279–283. (doi:10.1002/jcp.1041140305)

Rassendren F, Buell GN, Virginio C, Collo G, North RA & Surprenant A 1997 The permeabilizing ATP receptor, P2X7. Cloning and expression of a human cDNA. *Journal of Biological Chemistry* **272** 5482–5486. (doi:10.1074/jbc.272.9.5482)

Reyes JP, Sims SM & Dixon SJ 2011 P2 receptor expression, signaling and function in osteoclasts. *Frontiers in Bioscience* **3** 1101–1118. (doi:10.2741/214)

Roger S, Gillet L, Baroja-Mazo A, Surprenant A & Pelegrin P 2010 C-terminal calmodulin-binding motif differentially controls human and rat P2X7 receptor current facilitation. *Journal of Biological Chemistry* **285** 17514–17524. (doi:10.1074/jbc.M109.053082)

Rumney RM, Wang N & Gartland A 2010 The role of P2X (7) receptors in ATP release from osteoblasts. In *Purinergic Signalling*, pp 129–129. Springer.

Rumney RMH, Agrawal A, Shah K & Gartland A 2011 Fluid flow stimulates ATP release from human derived osteoclasts without changing resorption. Conference Abstract: 2011 Joint meeting of the Bone Research Society & the British Orthopaedic Research Society. *Frontiers in Endocrinology*. (doi:10.3389/conf.fendo.2011.02.00056)

Rumney RM, Sunters A, Reilly GC & Gartland A 2012*a* Application of multiple forms of mechanical loading to human osteoblasts reveals increased ATP release in response to fluid flow in 3D cultures and differential regulation of immediate early genes. *Journal of Biomechanics* **45** 549–554. (doi:10.1016/j.jbiomech.2011.11.036)

Rumney RMH, Wang N, Agrawal A & Gartland A 2012*b* Purinergic signalling in bone. *Frontiers in Endocrinology* **3** 116. (doi:10.3389/fendo.2012.00116)

Schafer R, Sedehizade F, Welte T & Reiser G 2003 ATP- and UTP-activated P2Y receptors differently regulate proliferation of human lung epithelial tumor cells. *American Journal of Physiology. Lung Cellular and Molecular Physiology* **285** L376–L385. (doi:10.1152/ajplung.00447.2002)

Shabbir M & Burnstock G 2009 Purinergic receptor-mediated effects of adenosine 5′-triphosphate in urological malignant diseases. *International Journal of Urology* **16** 143–150. (doi:10.1111/j.1442-2042.2008.02207.x)

Sivaramakrishnan V & Fountain SJ 2012 A mechanism of intracellular P2X receptor activation. *Journal of Biological Chemistry* **287** 28315–28326. (doi:10.1074/jbc.M112.372565)

Solle M, Labasi J, Perregaux DG, Stam E, Petrushova N, Koller BH, Griffiths RJ & Gabel CA 2001 Altered cytokine production in mice lacking P2X(7) receptors. *Journal of Biological Chemistry* **276** 125–132. (doi:10.1074/jbc.M006781200)

Stokes L & Surprenant A 2009 Dynamic regulation of the P2X4 receptor in alveolar macrophages by phagocytosis and classical activation. *European Journal of Immunology* **39** 986–995. (doi:10.1002/eji.200838818)

Sun D, Junger WG, Yuan C, Zhang W, Bao Y, Qin D, Wang C, Tan L, Qi B, Zhu D *et al*. 2013 Shockwaves induce osteogenic differentiation of human mesenchymal stem cells through ATP release and activation of P2X7 receptors. *Stem Cells* **31** 1170–1180. (doi:10.1002/stem.1356)

Syberg S, Petersen S, Beck Jensen JE, Gartland A, Teilmann J, Chessell I, Steinberg TH, Schwarz P & Jorgensen NR 2012*a* Genetic background strongly influences the bone phenotype of P2X7 receptor knockout mice. *Journal of Osteoporosis* **2012** 391097. (doi:10.1155/2012/391097)

Syberg S, Schwarz P, Petersen S, Steinberg TH, Jensen JE, Teilmann J & Jorgensen NR 2012*b* Association between P2X7 receptor polymorphisms and bone status in mice. *Journal of Osteoporosis* **2012** 637986. (doi:10.1155/2012/637986)

Takayanagi H, Kim S, Koga T, Nishina H, Isshiki M, Yoshida H, Saiura A, Isobe M, Yokochi T, Inoue J *et al*. 2002 Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. *Developmental Cell* **3** 889–901. (doi:10.1016/S1534-5807(02)00369-6)

Thimm D, Knospe M, Abdelrahman A, Moutinho M, Alsdorf BB, von Kugelgen I, Schiedel AC & Muller CE 2013 Characterization of new G protein-coupled adenine receptors in mouse and hamster. *Purinergic Signalling* **9** 415–426. (doi:10.1007/s11302-013-9360-9)

Toulme E, Garcia A, Samways D, Egan TM, Carson MJ & Khakh BS 2010 P2X4 receptors in activated C8-B4 cells of cerebellar microglial origin. *Journal of General Physiology* **135** 333–353. (doi:10.1085/jgp.200910336)

Vangsted AJ, Klausen TW, Gimsing P, Abildgaard N, Andersen NF, Gang AO, Holmstrom M, Gregersen H, Vogel U, Schwarz P *et al*. 2014 Genetic variants in the P2RX7 gene are associated with risk of multiple myeloma. *European Journal of Haematology* **93** 172–174. (doi:10.1111/ejh.12353)

Verkhratsky A & Burnstock G 2014 Biology of purinergic signalling: its ancient evolutionary roots, its omnipresence and its multiple functional significance. *BioEssays* **36** 697–705. (doi:10.1002/bies.201400024)

Virginio C, MacKenzie A, North RA & Surprenant A 1999 Kinetics of cell lysis, dye uptake and permeability changes in cells expressing the rat P2X7 receptor. *Journal of Physiology* **519** 335–346. (doi:10.1111/j.1469-7793.1999.0335m.x)

Volonte C, Apolloni S, Skaper SD & Burnstock G 2012 P2X7 receptors: channels, pores and more. *CNS & Neurological Disorders Drug Targets* **11** 705–721. (doi:10.2174/187152712803581137)

Wang L, Jacobsen SE, Bengtsson A & Erlinge D 2004*a* P2 receptor mRNA expression profiles in human lymphocytes, monocytes and CD34$^+$ stem and progenitor cells. *BMC Immunology* **5** 16. (doi:10.1186/1471-2172-5-16)

Wang Q, Wang L, Feng YH, Li X, Zeng R & Gorodeski GI 2004*b* P2X7 receptor-mediated apoptosis of human cervical epithelial cells. *American Journal of Physiology. Cell Physiology* **287** C1349–C1358. (doi:10.1152/ajpcell.00256.2004)

Wesselius A, Bours MJ, Henriksen Z, Syberg S, Petersen S, Schwarz P, Jorgensen NR, van Helden S & Dagnelie PC 2013 Association of P2X7 receptor polymorphisms with bone mineral density and osteoporosis risk in a cohort of Dutch fracture patients. *Osteoporosis International* **24** 1235–1246. (doi:10.1007/s00198-012-2059-x)

White N, Ryten M, Clayton E, Butler P & Burnstock G 2005 P2Y purinergic receptors regulate the growth of human melanomas. *Cancer Letters* **224** 81–91. (doi:10.1016/j.canlet.2004.11.027)

Wilson HL, Wilson SA, Surprenant A & North RA 2002 Epithelial membrane proteins induce membrane blebbing and interact with the P2X7 receptor C terminus. *Journal of Biological Chemistry* **277** 34017–34023. (doi:10.1074/jbc.M205120200)

Yoshida K, Oida H, Kobayashi T, Maruyama T, Tanaka M, Katayama T, Yamaguchi K, Segi E, Tsuboyama T, Matsushita M *et al*. 2002 Stimulation of bone formation and prevention of bone loss by prostaglandin E EP4 receptor activation. *PNAS* **99** 4580–4585. (doi:10.1073/pnas.062053399)

Zheng LM, Zychlinsky A, Liu CC, Ojcius DM & Young JDE 1991 Extracellular Atp as a trigger for apoptosis or programmed cell-death. *Journal of Cell Biology* **112** 279–288. (doi:10.1083/jcb.112.2.279)

Zippel N, Limbach CA, Ratajski N, Urban C, Luparello C, Pansky A, Kassack MU & Tobiasch E 2012 Purinergic receptors influence the differentiation of human mesenchymal stem cells. *Stem Cells and Development* **21** 884–900. (doi:10.1089/scd.2010.0576)

Received in final form 12 January 2015
Accepted 14 January 2015
Accepted Preprint published online 14 January 2015

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-14-0226

Published by Bioscientifica Ltd.