Biochemical and Biophysical Research Communications 494 (2017) 663–667

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

PYK2 mediates BzATP-induced extracellular matrix proteins synthesis

Go Torigoe [a], Mayu Nagao [b], Natsuko Tanabe [c, d, *], Soichiro Manaka [b], Taro Kariya [e], Takayuki Kawato [f, d], Jumpei Sekino [a], Shunichiro Kato [a], Masao Maeno [g], Naoto Suzuki [c, d], Noriyoshi Shimizu [e, h]

[a] Division of Applied Oral Science, Nihon University Graduate School of Dentistry, Tokyo, Japan
[b] Department of Periodontology, Nihon University School of Dentistry, Tokyo, Japan
[c] Department of Biochemistry, Nihon University School of Dentistry, Tokyo, Japan
[d] Division of Functional Morphology, Dental Research Center, Nihon University School of Dentistry, Tokyo, Japan
[e] Department of Orthodontics, Nihon University School of Dentistry, Tokyo, Japan
[f] Department of Oral Health Sciences, Nihon University School of Dentistry, Tokyo, Japan
[g] Nihon University, Tokyo, Japan
[h] Division of Clinical Research, Dental Research Center, Nihon University School of Dentistry, Tokyo, Japan

ARTICLE INFO

Article history:
Received 14 October 2017
Accepted 20 October 2017
Available online 20 October 2017

Keywords:
PYK2
BzATP
P2X7
Mechanical stimuli
Extracellular matrix proteins

ABSTRACT

Mechanical stimuli such as fluid shear and cyclic tension force induced extracellular adenosine triphosphate (ATP) release in osteoblasts. In particular, cyclic tension force-induced ATP enhances bone formation through P2X7 activation. Proline-rich tyrosine kinase 2 (PYK2) mediate osteoblasts differentiation is induced by mechanical stimuli. Furthermore, activation of PYK2 also was a response to integrin by mechanical stimuli. Extracellular matrix protein (ECMP)s, which are important factors for bone formation are expressed by osteoblasts. However, the effect of the interaction of 2'(3)-*O*-(4-Benzoylbenzoyl) adenosine-5'-triphosphate (BzATP), which is the agonist of the mechanosensitive receptor P2X7, with PYK2 on ECMP production is poorly understood. Thus, our purpose was to investigate the effects of PYK2 on BzATP-induced ECMP production in osteoblasts.

BzATP increased phospho-PYK2 protein expression on days 3 and 7 of culture. Furthermore, the PYK2 inhibitor PF431394 inhibited the stimulatory effect of BzATP on the expression of type I collagen, bone sialoprotein and osteocalcin expression. PF431396 did not inhibit the stimulatory effect of BzATP on osteopontin (OPN) mRNA expression.

These results suggest that mechanical stimuli activate P2X7 might induce ECMPs expression through PYK2 except in the case of OPN expression. Altogether, mechanical stimuli-induced ECMPs production might be implicated by extracellular ATP secretion or integrin via PYK2 activation.

1. Introduction

The homeostasis of skeletal bone is regulated by hormones, cytokines, and external factors such as mechanical stimuli. Mechanical stress is also a major factor affecting the amount and strength of the bone tissue required to maintain the bone mass in adults [1]. Furthermore, mechanical stimuli can be used to improve clinical treatment for conditions like bone fracture and orthodontic tooth movement.

Adenosine triphosphate (ATP) one of the nucleotides, is released by mechanical stimulation or inflammation that is mediated by autocrine/paracrine on osteoclasts and osteoblasts, which participate in bone remodeling [2]. ATP is promoted by pressure, stretch, and flow shear, as well as osmotic stress in various cell types [2,3]. ATP is a major of the regulator of osteoblasts response to mechanical stimuli [1,4]. Extracellular ATP is promoted by many of physiological responses through the activation of ATP-binding purinergic (P2) receptors such as P2Y family of G protein-coupled receptors and the P2X family of ligand-gated cation channels [5]. P2X3, P2X4, P2X5 and P2X7 are expressed in MC3T3-E1 cells [6], but P2X4 is not implicated in promoting bone formation by extracellular nucleotides [7]. However, the P2X7 receptor is expressed in response to fluid shearing stress, performed caused by mediate

* Corresponding author. Department of Biochemistry, Nihon University School of Dentistry, Tokyo, Japan.
E-mail address: tanabe.natsuko@nihon-u.ac.jp (N. Tanabe).

https://doi.org/10.1016/j.bbrc.2017.10.107
0006-291X/

pore formation in mouse calvarial cells. P2X7$^{-/-}$ mice also demonstrated decreased osteogenesis in response to mechanical loading in long bones; however, the deficiency of P2X7 did not affect the length of mouse long bones [8,9]. 2′(3)-*O*-(4-Benzoylbenzoyl) adenosine-5′-triphosphate (BzATP) is a specific antagonist of P2X7. Panupinthu et al., have been reported that BzATP induced bone formation through the production of lysophosphatidic acid and prostaglandin E_2 in mouse calvarial osteoblasts [10]. Our previous studies showed that mechanical stimuli induced the expression of extracellular matrix proteins (ECMPs) expression and osteogenesis through P2X7 activation in MC3T3-E1 cells *in vitro* [11,12]. These reports indicate that the P2X7 specific agonist BzATP, which is induced by mechanical stimuli might be a critical factor in osteogenesis.

Proline-rich tyrosine kinase 2 (PYK2) belongs to focal adhesion kinase subfamily of non-receptor tyrosine kinases. PYK2 is a large multidomain protein that contains an N-terminal FERM domain, a central catalytic domain, and a C-terminal segment that includes dual proline rich (Pr) subdomains and a focal adhesion targeting region [13,14]. PYK2 is related to a variety of proteins including p130CAS [15], Src [16], Cbl [17], integrins [16], gelsolin [18], and paxillin [19]. Integrins are activated by mechanical strain-induced ERK [20]. Integrins also mediated cell attachment through the activation of many intracellular signaling pathways; tyrosine phosphorylation cascades, calcium influx, inositol lipid turnover, and mitogen-activated protein kinase (MAPK) [21]. A previous study showed that the mechanical stimuli of tension force activate PYK2 implicating an intracellular Ca^{2+} dependence in osteoblasts [22]. However, the interaction of P2X7 agonist BzATP with PYK2 on the expression of ECMPs are elusive. Thus, our purpose was to investigate the effects of PYK2 on BzATP-induced ECMPs production.

2. Material and methods

2.1. Cell culture

The MC3T3-E1 mouse calvarial cell line obtained from Riken Bio Resource Center (Tsukuba, Japan) was used as osteoblast-like cells. Cells were maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO_2 in α-minimal essential medium (MEM; Gibco BRL, Rockville, MD, USA), containing 10% (v/v) heat-inactivated fetal bovine serum (HyClone Laboratories, Logan, UT, USA) and 1% (v/v) penicillin–streptomycin solution (Sigma-Aldrich, St. Louis, MO, USA). Cells were treated with 100 μM 2′(3)-*O*-(4-Benzoylbenzoyl) adenosine-5′-triphosphate (BzATP) (Sigma-Aldrich) or left untreated. The medium was replaced every 3 days.

2.2. Real-time polymerase chain reaction (real-time PCR)

Cells were seeded on 6-well plates and cultured for up to 14 days. Total RNA was isolated on days 3, 7, and 14 of culture using the RNeasy Mini Kit (QIAGEN, Valencia, CA, USA), and the RNA concentration was measured by Nano Drop 1000 (ND-1000; Thermo Fisher Scientific, Wilmington, DE, USA). Complementary DNA (cDNA) was synthesized from 0.5 μg of DNase-treated total RNA using the PrimeScript RT reagent kit (Takara Bio, Shiga, Japan), and the resultant cDNA was analyzed by real-time PCR using the SYBR Green kit (Takara Bio). The primer sequences are shown in Table 1. The PCR assays were performed with a Smart Cycler II instrument (Cepheid, Sunnyvale, CA, USA) and analyzed using Smart Cycler software. The cycling conditions included 35 cycles at 95 °C for 5 s and 60 °C for 20 s. All real-time PCR experiments were performed in triplicate, and the specificity of the amplified products was verified by melting curve analysis. The calculated values of the target gene expression were normalized to the level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which was used as an internal control [23].

2.3. Western blotting

Cells were cultured in serum-free medium after BzATP treatment and harvested 24 h later. The total protein concentrations in cell lysates were quantified, and 20 μg of protein from each sample was resolved using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes. The membrane was then treated with blocking reagent (1% (v/v) BSA) in Tris-buffered saline (TBS) (10 mM Tris, 145 mM NaCl, pH 7.4) for 18 h at 4 °C, washed in Tween 20-containing Tris-buffered saline (TBS) (10 mM Tris, 145 mM NaCl, pH 7.4) (TBS-Tween), and incubated with rabbit polyclonal IgG antibodies or mouse monoclonal IgG antibody, specific for PYK2, phospho-PYK2 (Cell Signaling technology, MA, USA), type I collagen (Col I), bone sialoprotein (BSP), osteocalcin (OCN), and β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) diluted 1:200 or 1:1000 in blocking reagent (1% (v/v) BSA) for 1 h at room temperature. β-actin was used as an internal standard. Membranes were then washed in TBS-Tween and incubated with HRP-conjugated secondary antibodies diluted 1:5000 in 1% blocking agent for 2 h at room temperature. Immunoreactive proteins were visualized using Image J, provided by the NIH.

2.4. Statistical analysis

Data represent the results of three independent experiments with samples that were prepared in triplicate. Each value represents the mean ± standard error (SEM). Differences between groups were evaluated with the one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test or two-way ANOVA with Bonferroni's multiple comparisons test. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. BzATP affects phospho-PYK2 protein expressions

To clarify the effect of PYK2 on BzATP in osteoblasts, we determined the protein expression of phospho-PYK2 (*p*-PYK2) on 3 and 7 days of culture. BzATP increased *p*-PYK2 compared to untreated control both 3 and 7 days of culture (Fig. 1).

3.2. Effects of PYK2 on BzATP-induced ECMPs mRNA expression

We investigated the effects of PYK2 on the BzATP-induced mRNA expression of ECMPs. BzATP increased mRNA levels of Col I

Table 1
PCR primers used in the experiments.

Target	Primers	GenBank Acc.
Col I	5′-TGGGCGCGGCTGGTATGAGTTC-3′ 5′-ACCCTGCTACGACAACGTGCC-3′	NM_007743.2
BSP	5′-AATTCTGACCCTCGTAGCCTTCATA-3′ 5′-GAGCCTCGTGGCGACACTTA-3′	NM_008318.3
OPN	5′-TACGACCATGAGATTGGCAGTGA-3′ 5′-TATAGGATCTGGGTGCAGGCTGTAA-3′	NM_009263.3
OCN	5′-AAGCAGGAGGGCAATAAGGT-3′ 5′-ACCCTGCTACGACAACGTGCC-3′	NM_007541.2
GAPDH	5′-AAATGGTGAAGGTCGGTGTG-3′ 5′-TGAAGGGGTCGTTGATGG-3′	NM_008084.2

Col I, Type I collagen; BSP, bone sialoprotein; OPN; osteopontin, OCN, osteocalcin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

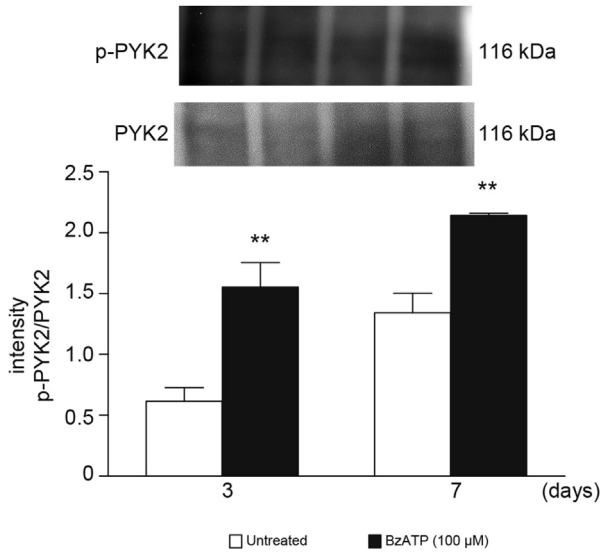

Fig. 1. Cells were stimulated with BzATP (100 μM) or left without stimulation (Untreated) and the protein expression of *p*-PYK2 (a), β-actin (b), was determined on days 3 and 7 of culture using Western Blot, histograms show the intensity of western blotting bands under each condition. Data are expressed as the mean ± SEM of three independent experiments performed in triplicate; $^{**}p < 0.01$, vs. Untreated.

by day 3 and 7 of culture (by 2.0 and 1.68-fold, respectively) compared to untreated control (Fig. 2a). mRNA expression of BSP was increased by BzATP on days 3, 7 and 14 of culture (by 1.5, 1.7 and 3.8-fold, respectively) compared to untreated control (Fig. 2b). OPN mRNA level was increased by BzATP on day 7 of culture (by 1.42-fold) compared to untreated control (Fig. 2c). OCN mRNA level was also increased by BzATP on days 7 and 14 of culture (by 5.57 and 6-fold, respectively) compared to untreated control (Fig. 2d). PYK2 inhibitor PF431396 blocked stimulatory effect of BzATP on mRNA expression of Col I on days 3 and 7 (by 0.66 and 0.53-fold, respectively), BSP on day 3 (by 0.85-fold) and OCN on day 7 (by 0.08-fold). However, OPN did not have the significantly stimulatory effect of PF431396 (Fig. 2a–d).

3.3. Effects of PYK2 on BzATP-induced ECMPs protein expression

We next determined the effects of PYK2 on BzATP-induced ECMPs protein expression. BzATP-induced Col I and BSP protein expression was inhibited by PF431396 on day 3 (by 0.58 and 0.86-fold, respectively) (Fig. 3a and b). The protein expression of OCN which induced BzATP was also inhibited by PF431396 on day 7 (by 0.38-fold, respectively) (Fig. 3c). We did not determine OPN protein expression because the mRNA level of OPN was unaffected by PYK2 inhibition because of PF431396 (Fig. 1c).

The protein levels of the ECMPs also exhibited the similar effects as mRNA levels (Fig. 3).

4. Discussion

BzATP is a specific agonist of P2X7 that induces the opening of P2X7 channels, by causing the elevation of intracellular Ca^{2+} and depolarization of the plasma membrane [24]. P2X7 receptor is a nonselective cation channel permeable to Na^{+}, K^{+} and Ca^{2+}. Previous studies demonstrated that BzATP or a high concentration of ATP increases cytosolic Ca^{2+} concentration through P2X7 activation [10,25]. Functional P2X7 receptors can be expressed in osteoblasts both *in situ* and *in vitro* [10]. In previous studies, $P2X7^{-/-}$ mice had reduced osteogenesis in load-bearing bones, which suggests that

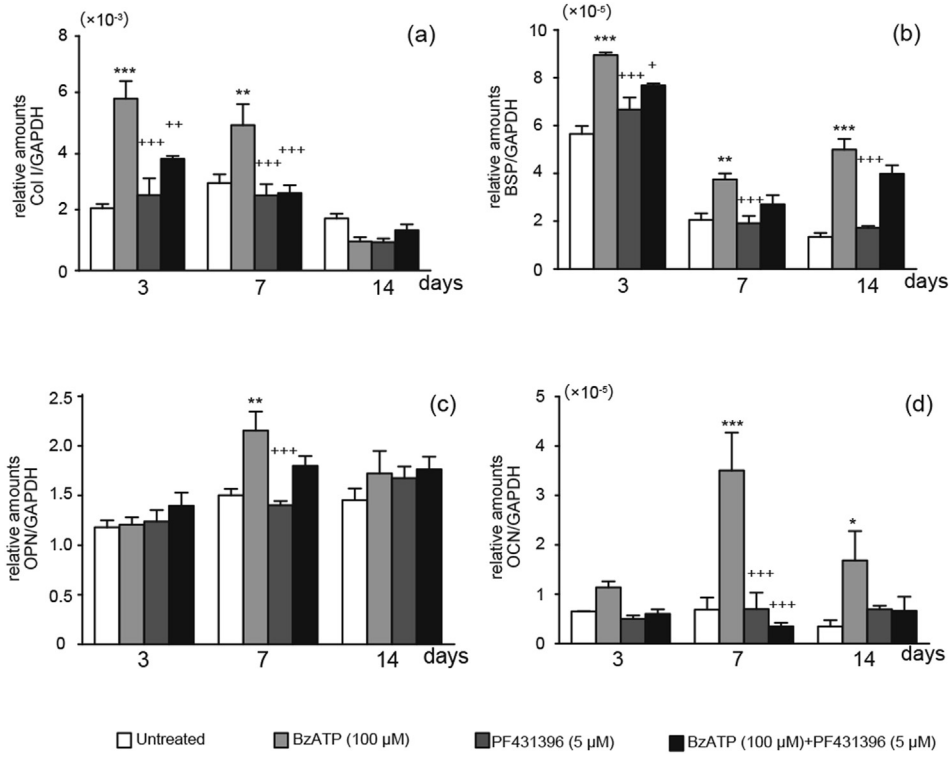

Fig. 2. Cells were stimulated with BzATP (100 μM) and/or PYK2 inhibitor PF431396 (5 μM) or left without stimulation (Untreated) and the gene expression of Col I (a), BSP (b), OPN (c) and OCN (d) was determined on days 3, 7 and 14 of culture using real-time PCR. Data are expressed as the mean ± SEM of three independent experiments performed in triplicate; $^{**}p < 0.01$, $^{***}p < 0.001$, vs. Untreated, $++p < 0.01$, $+++p < 0.001$, vs BzATP.

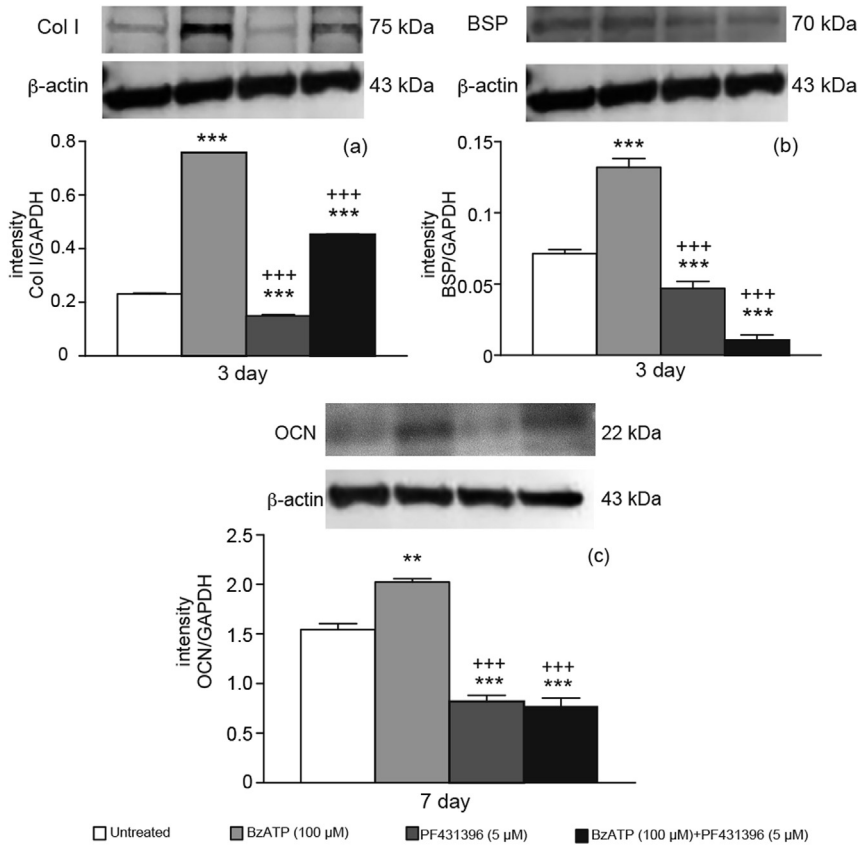

Fig. 3. Cells were stimulated with BzATP (100 μM) and/or PYK2 inhibitor PF431396 (5 μM) or left without stimulation (Untreated) and the protein expression of Col I (a), BSP (b), OPN (c) and OCN (d) was determined on days 3, 7 and 14 of culture using Western Blot; histograms show the intensity of western blotting bands under each condition. Data are expressed as the mean ± SEM of three independent experiments performed in triplicate; $^{*}p < 0.05$,$^{**}p < 0.01$, $^{***}p < 0.001$, vs. Untreated, $++p < 0.01$, $+++p < 0.001$, vs BzATP.

the activation of P2X7 is responsive to mechanical stress in the skeletal bone [8,9]. However, Orriss et al., reported that the activation of P2X7 receptor reduced bone formation in primary rat osteoblasts [7]. These findings indicate an opposite effect on bone formation because of P2X7, but this study only investigated this effect in rat primary osteoblasts.

Mechanical stimuli which include pressure, stretch, fluid shear and osmotic stress induces extracellular ATP release in various cell types such as osteoblasts [2,3]. Extracellular ATP release is also stimulated via both constitutive and inductive mechanisms [26]. It has been demonstrated that the secretion of extracellular ATP promotes osteogenesis in rodent calvarial cells *in vitro* [10,27]. Our previous studies demonstrated that cyclic tension force and low-intensity pulsed ultrasound induced extracellular ATP release, which enhanced bone formation through P2X7 in MC3T3-E1 cells [11,12].

Antisense depletion of PYK2 [28], which is not the expression of a kinase inactive kinase mutant [29] inhibited osteoclastogenesis that indicates that the catalytic activity of PYK2 may be dispensable. A mechanical transducer of integrin was mediated by PYK2-Ca^{2+} pathway in osteoblasts [20]. Moreover, periodic mechanical stress induces chondrocyte proliferation and matrix synthesis via the Calmodulin-dependent kinase II (CaMKII)-PYK2 pathway [30]. CaMKII is a multifunctional serine/threonine kinase and has been confirmed to be a critical regulator of Ca^{2+} in various signaling pathways including the nuclear factor of activated T-cells (NFAT) c1 pathway. These findings indicate that both PYK2 and CaMKII are involved in the Ca^{2+}-dependent pathway. Moreover, Grol et al., have been reported that BzATP induced Ca^{2+}-NFATc1 activation, and Panupinthu et al. have also indicated that BzATP increases gene expression of ECMPs in osteoblasts [10,25]. Thus, we investigated the effects of PYK2 on BzATP-induced ECMPs expression. As a result, we observed that BzATP increased *p*-PYK2 on days 3 and 7 of culture. Furthermore, the PYK2 inhibitor PF431396 inhibited the stimulatory effect of BzATP on the expression of type I collagen (Col I), bone sialoprotein (BSP) and osteocalcin (OCN) expression (Figs. 2 and 3).

Col I acts as a scaffold for the nucleation of hydroxyapatite crystals through the calcification process, and therefore is a major protein constituent of ECM in bone tissue. BSP, OPN, and OCN are non-collagenous matrix proteins that have important roles in the organization of the collagen matrix [31]. In addition, BSP have a role of a nucleation center for the hydroxyapatite formation in the mineralized nodule formation in bone [32]. OCN has small γ-carboxyglutamate protein, that is selectively expressed by osteoblasts [33]. OCN is also the most abundant non-collagenous bone matrix protein [34] and is a major marker of differentiated osteoblasts. The functions of OPN are diverse and directly related to bone formation and remodeling, as with fundamental roles in host defense and tissue repair [35]. A previous study reported that mechanical stimuli induce OPN mRNA expression in osteoblasts, whereas PYK2 did not mediate this expression [36]. In our present study, BzATP induced gene expression of OPN, whereas PF431396 did not affect OPN mRNA levels, which is consistent with the results of previous study. BzATP-induced OPN might be regulated by another signal molecule such as focal adhesion kinase (FAK), as described in a previous report [36]. Taken together, these our results suggest that mechanical stimuli-induced ECMPs production might be linked to extracellular ATP secretion and/or integrin via PYK2 activation.

In summary, the P2X7 specific agonist of BzATP enhanced *p*-PYK2. PYK2 inhibitor of PF431396 blocked the stimulatory effect of BzATP on ECMPs production. These results showed that the activation of P2X7 by mechanical stimuli might induce ECMPs expression, except in case of OPN.

Authors roles

GT, NT, and NS conceived and designed the experiments. GT, JS, and SM performed Cell culture experiments. MN, SK and GT performed Real-time PCR experiments. JS, and TK helped in Western blotting. MN, NT, NS, and MM analyzed the data. JS, SK, and TK helped in providing regent/materials/analysis tools. GT, NT, and NS wrote the paper.

Conflicts of interest

There are no conflicts of interest to declare.

Ethical Approval and Consent to participate

All study participants provided informed consent, and the study design was approved by the appropriate ethics review boards.

Consent for publication

All the authors have approved the manuscript and agree with submission to your esteemed journal.

Acknowledgments

This study was supported by a grant from sato fund and Uemura fund, Nihon University school of Dentistry, Grant from Dental Research Center, Nihon University Multidisciplinary Research Grant for 2017 and 2018 and Grant-in-Aid for Scientific Research (C) (15K11354), and Grant-in-Aid for Research Activity start-up (15H06646), Japan Society for the Promotion of Science.

References

[1] A.G. Robling, A.B. Castillo, C.H. Turner, Biomechanical and molecular regulation of bone remodeling, Annu. Rev. Biomed. Eng. 8 (2006) 455–498.
[2] D.C. Genetos, D.J. Geist, D. Liu, H.J. Donahue, R.L. Duncan, Fluid shear-induced ATP secretion mediates prostaglandin release in MC3T3-E1 osteoblasts, J. Bone Min. Res. 20 (2005) 41–49.
[3] H.A. Praetorius, J. Leipziger, ATP release from non-excitable cells, Purinergic Signal. 5 (2009) 433–446.
[4] S.J. Dixon, S.M. Sims, P2 purinergic receptors on osteoblasts and osteoclasts: potential targets for drug development, Drug Dev. Res. 49 (2000) 187–200.
[5] G. Burnstock, Physiology and pathophysiology of purinergic neurotransmission, Physiol. Rev. 87 (2007) 659–797.
[6] J. Qi, L. Chi, J. Faber, B. Koller, A.J. Banes, ATP reduces gel compaction in osteoblast-populated collagen gels, J. Appl. Physiol. 102 (2007) 1152–1160.
[7] I.R. Orriss, M.L. Key, A. Brandao-Burch, J.J. Patel, G. Burnstock, T.R. Arnett, The regulation of osteoblast function and bone mineralisation by extracellular nucleotides: the role of p2x receptors, Bone 51 (2012) 389–400.
[8] J. Li, D. Liu, H.Z. Ke, R.L. Duncan, C.H. Turner, The P2X7 nucleotide receptor mediates skeletal mechanotransduction, J. Biol. Chem. 280 (2005) 42952–42959.
[9] H.Z. Ke, H. Qi, A.F. Weidema, Q. Zhang, N. Panupinthu, D.T. Crawford, W.A. Grasser, V.M. Paralkar, M. Li, L.P. Audoly, C.A. Gabel, W.S. Jee, S.J. Dixon, S.M. Sims, D.D. Thompson, Deletion of the P2X7 nucleotide receptor reveals its regulatory roles in bone formation and resorption, Mol. Endocrinol. 17 (2003) 1356–1367.
[10] N. Panupinthu, J.T. Rogers, L. Zhao, L.P. Solano-Flores, F. Possmayer, S.M. Sims, S.J. Dixon, P2X7 receptors on osteoblasts couple to production of lysophosphatidic acid: a signaling axis promoting osteogenesis, J. Cell Biol. 181 (2008) 859–871.
[11] T. Kariya, N. Tanabe, C. Shionome, S. Manaka, T. Kawato, N. Zhao, M. Maeno, N. Suzuki, N. Shimizu, Tension force-induced ATP promotes osteogenesis through P2X7 receptor in osteoblasts, J. Cell Biochem. 116 (2015) 12–21.
[12] S. Manaka, N. Tanabe, T. Kariya, M. Naito, T. Takayama, M. Nagao, D. Liu, K. Ito, M. Maeno, N. Suzuki, M. Miyazaki, Low-intensity pulsed ultrasound-induced ATP increases bone formation via the P2X7 receptor in osteoblast-like MC3T3-E1 cells, FEBS Lett. 589 (2015) 310–318.
[13] H. Avraham, S.Y. Park, K. Schinkmann, S. Avraham, RAFTK/Pyk2-mediated cellular signalling, Cell Signal 12 (2000) 123–133.
[14] S. Han, A. Mistry, J.S. Chang, D. Cunningham, M. Griffor, P.C. Bonnette, H. Wang, B.A. Chrunyk, G.E. Aspnes, D.P. Walker, A.D. Brosius, L. Buckbinder, Structural characterization of proline-rich tyrosine kinase 2 (PYK2) reveals a unique (DFG-out) conformation and enables inhibitor design, J. Biol. Chem. 284 (2009) 13193–13201.
[15] P.T. Lakkakorpi, I. Nakamura, R.M. Nagy, J.T. Parsons, G.A. Rodan, L.T. Duong, Stable association of PYK2 and p130(Cas) in osteoclasts and their co-localization in the sealing zone, J. Biol. Chem. 274 (1999) 4900–4907.
[16] L.T. Duong, P.T. Lakkakorpi, I. Nakamura, M. Machwate, R.M. Nagy, G.A. Rodan, PYK2 in osteoclasts is an adhesion kinase, localized in the sealing zone, activated by ligation of alpha(v)beta3 integrin, and phosphorylated by src kinase, J. Clin. Invest 102 (1998) 881–892.
[17] A. Sanjay, A. Houghton, L. Neff, E. DiDomenico, C. Bardelay, E. Antoine, J. Levy, J. Gailit, D. Bowtell, W.C. Horne, R. Baron, Cbl associates with Pyk2 and Src to regulate Src kinase activity, alpha(v)beta(3) integrin-mediated signaling, cell adhesion, and osteoclast motility, J. Cell Biol. 152 (2001) 181–195.
[18] Q. Wang, Y. Xie, Q.S. Du, X.J. Wu, X. Feng, L. Mei, J.M. McDonald, W.C. Xiong, Regulation of the formation of osteoclastic actin rings by proline-rich tyrosine kinase 2 interacting with gelsolin, J. Cell Biol. 160 (2003) 565–575.
[19] M. Pfaff, P. Jurdic, Podosomes in osteoclast-like cells: structural analysis and cooperative roles of paxillin, proline-rich tyrosine kinase 2 (Pyk2) and integrin alphaVbeta3, J. Cell Sci. 114 (2001) 2775–2786.
[20] C. Schmidt, H. Pommerenke, F. Durr, B. Nebe, J. Rychly, Mechanical stressing of integrin receptors induces enhanced tyrosine phosphorylation of cytoskeletally anchored proteins, J. Biol. Chem. 273 (1998) 5081–5085.
[21] S.K. Alahari, P.J. Reddig, R.L. Juliano, Biological aspects of signal transduction by cell adhesion receptors, Int. Rev. Cytol. 220 (2002) 145–184.
[22] N. Boutahar, A. Guignandon, L. Vico, M.H. Lafage-Proust, Mechanical strain on osteoblasts activates autophosphorylation of focal adhesion kinase and proline-rich tyrosine kinase 2 tyrosine sites involved in ERK activation, J. Biol. Chem. 279 (2004) 30588–30599.
[23] Y. Takahashi, H. Tanaka, K. Nakai, S. Kitami, F. Murakami, T. Morita, N. Tanabe, T. Kawato, M. Maeno, RANKL induces IL-18 binding protein expression in RAW264.7 cells, J. Hard Tissue Biol. 25 (2016) 173–180.
[24] P. Pelegrin, A. Surprenant, Pannexin-1 mediates large pore formation and interleukin-1beta release by the ATP-gated P2X7 receptor, EMBO J. 25 (2006) 5071–5082.
[25] M.W. Grol, A. Pereverzev, S.M. Sims, S.J. Dixon, P2 receptor networks regulate signaling duration over a wide dynamic range of ATP concentrations, J. Cell Sci. 126 (2013) 3615–3626.
[26] L.C. Gerstenfeld, S.D. Chipman, J. Glowacki, J.B. Lian, Expression of differentiated function by mineralizing cultures of chicken osteoblasts, Dev. Biol. 122 (1987) 49–60.
[27] S.V. Komarova, F.I. Ataullakhanov, R.K. Globus, Bioenergetics and mitochondrial transmembrane potential during differentiation of cultured osteoblasts, Am. J. Physiol. Cell Physiol. 279 (2000) C1220–C1229.
[28] L.T. Duong, I. Nakamura, P.T. Lakkakorpi, L. Lipfert, A.J. Bett, G.A. Rodan, Inhibition of osteoclast function by adenovirus expressing antisense protein-tyrosine kinase 2, J. Biol. Chem. 276 (2001) 7484–7492.
[29] P.T. Lakkakorpi, A.J. Bett, L. Lipfert, G.A. Rodan, L.T. Duong, PYK2 autophosphorylation, but not kinase activity, is necessary for adhesion-induced association with c-Src, osteoclast spreading, and bone resorption, J. Biol. Chem. 278 (2003) 11502–11512.
[30] W. Liang, Z. Li, Z. Wang, J. Zhou, H. Song, S. Xu, W. Cui, Q. Wang, Z. Chen, F. Liu, W. Fan, Periodic mechanical stress INDUCES chondrocyte proliferation and matrix synthesis via CaMKII-mediated Pyk2 signaling, Cell Physiol. Biochem. 42 (2017) 383–396.
[31] A.L. Boskey, Mineral-matrix interactions in bone and cartilage, Clin. Orthop. Relat. Res. (1992) 244–274.
[32] G.K. Hunter, H.A. Goldberg, Nucleation of hydroxyapatite by bone sialoprotein, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 8562–8565.
[33] G. Boivin, G. Morel, J.B. Lian, C. Anthoine-Terrier, P.M. Dubois, P.J. Meunier, Localization of endogenous osteocalcin in neonatal rat bone and its absence in articular cartilage: effect of warfarin treatment, Virchows Arch. A Pathol. Anat. Histopathol. 417 (1990) 505–512.
[34] G. Wolf, Function of the bone protein osteocalcin: definitive evidence, Nutr. Rev. 54 (1996) 332–333.
[35] J. Sodek, B. Ganss, M.D. McKee, Osteopontin, Crit. Rev. Oral Biol. Med. 11 (2000) 279–303.
[36] S.R. Young, J.M. Hum, E. Rodenberg, C.H. Turner, F.M. Pavalko, Non-overlapping functions for Pyk2 and FAK in osteoblasts during fluid shear stress-induced mechanotransduction, PLoS One 6 (2011) e16026.