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Lysophosphatidic acid induces osteocyte dendrite outgrowth

Sue A. Karagiosis, Norman J. Karin *

Cell Biology and Biochemistry Group, Pacific Northwest National Laboratory, Richland, WA 99352, USA

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Abstract

Osteocytes elaborate an extensive mechanosensory network in bone matrix and communicate intercellularly via gap junctions established at dendrite termini. We developed a method to measure osteocyte dendritogenesis *in vitro* using a modified transwell assay and determined that the lipid growth factor lysophosphatidic acid (LPA) is a potent stimulator of dendrite outgrowth in MLO-Y4 osteocytes. The stimulatory effects were dose-dependent with maximal outgrowth observed within a physiological range of LPA. LPA-treated osteocytes exhibited distinct rearrangements of the actin cytoskeleton and a more stellate morphology than control cells. LPA also promoted osteocyte chemotaxis, suggesting a shared molecular mechanism between dendrite outgrowth and cell motility. The LPA-induced increase in dendrite formation was blocked by the specific LPA-receptor antagonist Ki16425 and by pertussis toxin. Bone cells *in vivo* encounter platelet-derived LPA in regions of bone damage, and we postulate that this lipid factor is important for re-establishing osteocyte connectivity during fracture repair.

Keywords: Osteocytes; Dendrite; Lysophosphatidic acid; LPA receptors; Cytoskeleton

Osteocytes arise from bone-forming osteoblasts that become encased in the mineralized extracellular matrix where they function as an intercellular communication network [1]. In contrast to the cuboidal morphology of their progenitor cells, mature osteocytes have multiple thin membrane extensions (dendrites). The dendritic processes extend through narrow bone channels (canaliculi) and are interconnected via gap junctions, leading to the formation of a functional syncytium. The function of osteocytes *in vivo* has been compared to the role of neuronal cells in sensing and transmitting physiological signals [2,3].

The osteocyte network has an essential role in the response of bone to physical strain, a process that is essential for normal bone mass homeostasis [4], and losses of connectivity among osteocytes were observed in association with osteoporosis, osteomalacia and osteoarthritis [1]. Osteocyte dendrites also form junctional contacts with osteoblasts, presumably to modulate bone forming activity [5]. Additional evidence suggests that osteocytes regulate bone-resorbing osteoclast activity via a mechanism that requires cell–cell contact [6].

Little is known regarding how osteocytes elaborate dendritic processes, establish junctional connections and transduce mechanical stimuli. The actin cytoskeleton is essential for the establishment and maintenance of osteocyte processes, and osteocytes subjected to physiological levels of mechanical strain exhibit robust changes in F-actin architecture [7,8]. Recent evidence revealed that dendrite length was increased in response to mechanical strain, and that osteocyte membrane processes proteolytically degrade the extracellular matrix (ECM) [9,10]. Thus, the generation of the osteocyte network appears to be an active and dynamic process and not simply a passive burial of membrane extensions during ECM deposition by neighboring osteoblasts [9,10]. Given the importance of the osteocyte network to bone health, it will be valuable to identify the molecular mechanisms essential for dendrite formation.

* Corresponding author. Fax: +1 509 376 6767.
E-mail address: Norman.Karin@pnl.gov (N.J. Karin).

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The role of LPA in the regulation of osteocyte function has not been unexplored. We and others have found that osteoblasts, the precursors to osteocytes, are highly responsive to the water-soluble lipid growth factor lysophosphatidic acid (LPA), exhibiting chemotaxis, increased proliferation, elevated resistance to apoptosis, activation of MAP kinases, and acute elevations in cytosolic Ca^{2+} [11–15]. LPA-treated MC3T3-E1 osteoblastic cells develop long actin-rich membrane extensions that resemble osteocyte dendritic processes [15]. The effects of this growth factor on the osteoblasts led us to predict that osteocytes would be target cells for LPA, and that this lipid would increase the outgrowth of osteocyte dendrites. We report here the development of an assay to measure osteocyte dendrite outgrowth *in vitro* and the demonstration that LPA induced a dose-dependent increase in membrane process extension. This is the first evidence for the regulation of osteocyte dendricity by a soluble factor. We hypothesize that LPA has a role in regulating osteocyte dendricity and mechanosensation *in vivo*.

Materials and methods

Cell culture and reagents. MLO-Y4 osteocyte-like cells [16], a gift from Dr. Lynda Bonewald (University of Missouri-Kansas City), were grown on gelatin-coated plates in αMEM (Cellgro) containing 5% fetal bovine serum and 5% donor calf serum (Valley Biomedical) in a humidified 5% CO_2/95% air atmosphere at 37 °C. Where indicated, cells were serum-starved by incubation in αMEM containing 0.1% BSA (αMEM/BSA). LPA (1-oleoyl-2-hydroxy-sn-3-glycerol-3-phosphate; Biomol) was added to cells from aqueous stock solutions. The bovine serum albumin (BSA) used in this study was essentially fatty acid-free (MP Biomedicals). Ki16425 and pertussis toxin were purchased from Sigma.

Osteocyte dendrite outgrowth and chemotaxis assays. The generation of osteocyte membrane processes was measured using a modification of a transwell assay designed to quantify neuronal outgrowth [17]. FluoroBlok cell culture inserts (1 μm pores; BD Biosciences) were coated by immersion in sterile 0.1% bovine gelatin at room temperature for 2–4 h. The coated inserts were placed into wells of 24-well plates containing 1 ml αMEM/BSA or αMEM/BSA with LPA at the concentrations specified. MLO-Y4 cells were suspended using a protease-free releasing agent (CellStripper; Mediatech), counted with a Coulter counter, centrifuged and suspended in αMEM/BSA with or without LPA receptor inhibitors. The cell suspension (100,000 cells in 300 μl) was added to the upper chambers, and the assembly was incubated in a humidified 5% CO_2 atmosphere at 37 °C for 16–18 h. The inserts were transferred into wells containing 3 μg/ml calcein acetoxymethyl ester (Molecular Probes) in Hank's-buffered saline solution (Gibco) and incubated for 30 min at 37 °C. The inserts then were placed in wells containing phosphate-buffered saline, and dendrite outgrowth was quantified as calcein fluorescence on the undersides of the opaque insert membranes using a CytoFluor 4000 (Applied Biosystems) set at 485 nm excitation and 530 nm emission in the bottom-read mode. Data were evaluated statistically using Student's two-tailed *t*-test, and results were considered to be significant if $p < 0.05$. Images of dendrites on the undersides of the transwell membranes were captured using a Leica DM IRE2 epifluorescence microscope and a 20X PL Fluotar objective. To quantify the effect of LPA on MLO-Y4 cell migration, assay conditions were identical to those described above except that gelatin-coated transwell membranes with 3 μm pores were used.

Alexa Fluor 568-labeled phalloidin (Molecular Probes) was used to label F-actin in paraformaldehyde-fixed cells as described previously [15]. To confirm that entire osteocytes cannot cross 1 μm pores, transwell membranes were excised and the nuclei of methanol-fixed LPA-treated cells were stained with 1 μM SYTOX Green (Molecular Probes). The membranes were mounted in Prolong Gold antifade mounting medium (Molecular Probes) between two glass coverslips which allowed both sides of the membranes to be viewed individually. Nuclear and F-actin images

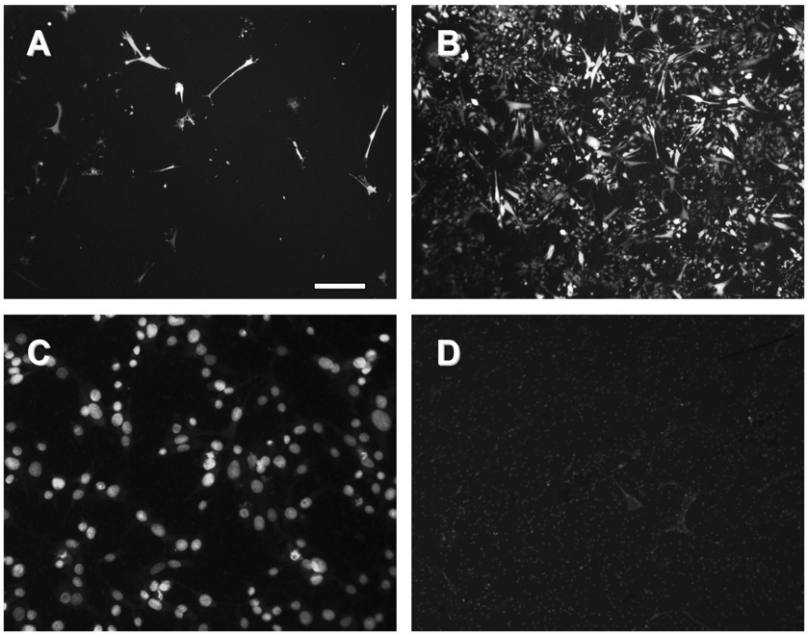

Fig. 1. LPA-induced MLO-Y4 dendrite outgrowth measured in a transwell assay system. Cells were incubated for 16 h in transwell inserts immersed in αMEM/BSA (A) or αMEM/BSA containing 1.0 μM LPA (B–D). (A,B) Undersides of transwell chamber membranes with calcein-stained osteocyte dendrites from control and LPA-treated cells, respectively. MLO-Y4 osteocytes do not migrate through the 1 μm pores of the transwell inserts. LPA-treated osteocytes on the upper (C) and lower (D) surfaces of a transwell chamber membrane were methanol-fixed and stained with SYTOX Green to label nuclei. No nuclei were detected on the underside of the membrane. Scale bar: 25 μm.

were captured using a Nikon Eclipse TE300 epifluorescence microscope and 20X and 60X Plan Fluor oil-immersion objectives, respectively.

Results

Osteocyte dendricity typically is quantified by the morphometric analysis of light or electron micrographs [10,18]. As a less laborious and more readily quantifiable alternative, we developed a transwell assay approach, based on a method for the study of neuronal membrane extension [17] and similar to the technique we employed to measure osteoblast chemotaxis [15]. In this assay, dendrite mass is determined from the fluorescence of calcein-labeled membrane processes that extend through 1 μm pores, while fluorescence associated with the cell bodies is excluded by the opaque FluoroBlok membranes. Few fluorescent dendrites were observed when osteocytes were incubated in serum-free medium in the absence of LPA (Fig. 1A). In contrast, a profusion of dendritic processes were present on the undersides of the transwell membranes when cells were treated with 1.0 μM LPA (Fig. 1B). To determine whether entire osteocytes were able to penetrate the pores, LPA-treated cells were fixed and stained with SYTOX Green to label nuclei. After incubation for 16 h in 1.0 μM LPA, many labeled nuclei were observed on the upper surfaces of the opaque transwell membranes where they were seeded (Fig. 1C) while no nuclei were detected on the undersides (Fig. 1D), confirming that only dendrites were able to pass through the 1 μm pores. We next measured the dose dependence of LPA-stimulated dendrite formation and found that this lipid growth factor initiated outgrowth at all concentrations tested (0.01–10 μM) (Fig. 2A). The maximum effect on dendricity was observed in cells treated with 1.0 μM, which is in accordance with the physiological levels of this lipid factor (1–5 μM) measured in serum [19].

We postulated that commonalities exist between the mechanisms that regulate osteocyte dendricity and those controlling cell motility. Therefore, we ascertained whether LPA, a potent chemotactic agent for osteoblasts [15], would stimulate the migration of MLO-Y4 cells through transwell membranes with 3 μm pores, which are sufficiently large for whole osteocytes to penetrate (data not shown). LPA induced MLO-Y4 cell chemotaxis over a concentration range of 0.01–10 μM with a dose dependence essentially identical to that of dendrite outgrowth (Fig. 2B). As with LPA-elicited dendrite formation, a maximal induction of cell migration was observed at 1.0 μM.

Dendrite formation requires alterations in the osteocyte cytoskeleton [8]. In contrast to control (untreated) osteocytes which contained relatively few F-actin stress fibers (Fig. 3A), many cells exhibited increased stress fiber formation after 1 h of LPA exposure (Fig. 3B). After 6 h of LPA treatment (Fig. 3C) numerous osteocytes exhibited a stellate morphology with F-actin-rich membrane protrusions. This highly dendritic morphology was even more pronounced after 16 h in LPA-containing medium (Fig. 3D).

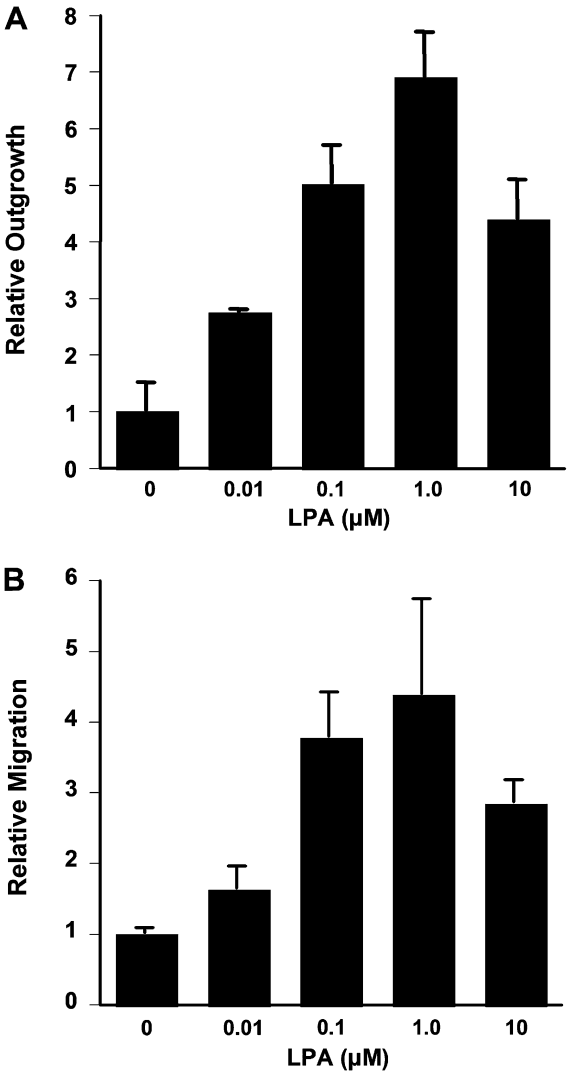

Fig. 2. Dose-dependent stimulation by LPA of osteocyte outgrowth and chemotaxis. MLO-Y4 cell dendrite formation and migration were quantified as described in Materials and methods. Data from duplicate wells (error bars = SE) were normalized to the outgrowth or migration in the absence of LPA. (A) Osteocyte outgrowth in LPA-treated cells was significantly greater than in untreated cells at all LPA concentrations tested ($p = 0.012$–0.039). (B) LPA significantly stimulated MLO-Y4 cell chemotaxis at each concentration ($p = 0.001$–0.029).

The effects of LPA on target cells are mediated by a family of G protein-coupled receptors that includes at least four members, LPA_1–LPA_4 [20]. We measured LPA receptor expression in MLO-Y4 cells using RT-PCR and found that cDNAs encoding LPA_1 and LPA_2 receptors were easily amplified. LPA_4 transcripts were present at much lower levels, and LPA_3 mRNA was barely detectable (Fig. 4A). This pattern is similar to the expression of LPA receptor transcripts in MC3T3-E1 osteoblasts [15]. We previously employed Ki16425, an inhibitor of LPA_1 and LPA_3 receptors [21], to demonstrate a functionally dominant role for LPA_1 in LPA-induced osteoblast chemotaxis [15]. Similar to the effect of this compound on osteoblast motility, Ki16425 inhibited the ability of LPA to initiate osteocyte

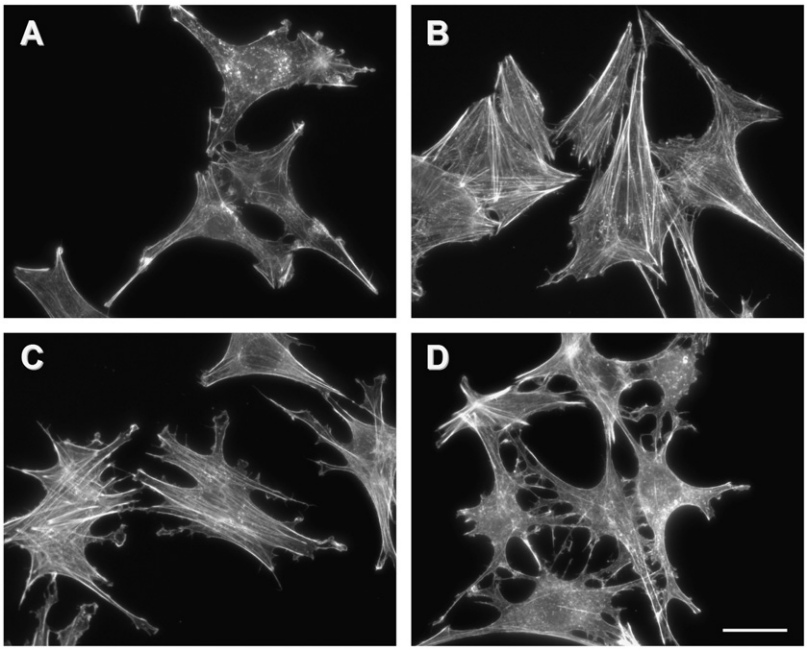

Fig. 3. LPA elicits actin reorganization in osteocytes. Epifluorescence micrographs show F-actin visualized with Alexa Fluor 568-phalloidin. Cells were serum-deprived in αMEM/BSA for 16 h before the addition of 1.0 μM LPA for (A) 0 h, (B) 1 h, (C) 6 h and (D) 16 h. Scale bar: 25 μm.

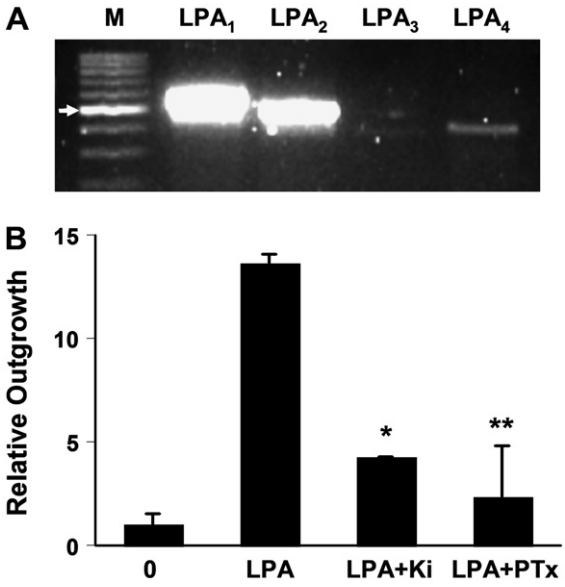

Fig. 4. Receptor-coupled stimulation of dendrite outgrowth in LPA-treated osteocytes. (A) The detection of transcripts encoding the four known LPA receptors was accomplished using RT-PCR as described previously [15]. Equal volumes from RT-PCR reactions were analyzed by electrophoresis through a 1.2% agarose gel. Expected amplimer sizes were: LPA_1, 573 base pairs (bp); LPA_2, 478 bp; LPA_3, 381 bp; and LPA_4, 409 bp. M, markers (100 bp ladder); the 500 bp marker is indicated with an arrow. (B) Dendrite formation was quantified as in Fig. 2. Data from duplicate wells (error bars, SE) were normalized to the outgrowth in the absence of 1.0 μM LPA. Ki, Ki16425; PTx, pertussis toxin. $^{*}p = 0.001$ vs. LPA alone; $^{**}p = 0.0236$ vs. LPA alone.

dendrite formation (Fig. 4B). LPA-induced dendrite outgrowth also was impaired if the cells were treated with the $G_{i/o}$ inhibitor pertussis toxin (Fig. 4B).

Discussion

The differentiation of osteoblasts into osteocytes during bone formation is accompanied by a 70% reduction in cell body volume due to the redistribution of mass to the dendritic processes [22]. Our results demonstrate that the lipid growth factor LPA stimulated dendritogenesis in MLO-Y4 osteocytes. The mechanisms by which dendrites form *in vivo* are not known, but dendrite length was influenced by mechanical strain [10], and recent imaging data revealed dynamic dendrite retraction and extension in GFP-labeled osteocytes in calvarial organ culture [23]. These observations suggest that osteocyte outgrowth is an actively regulated process. The arborization of osteocytes *in vivo* is asymmetrical: the length and number of dendrites facing the bone vascular space is greater than those oriented in the opposite direction [22]. The mechanisms by which this polarity is established is unknown but may include the response of osteocytes to humoral factors emanating from the vasculature [2]. Our data showing that the serum factor LPA induces dendrite outgrowth is consistent with this postulate. Interestingly, LPA is a potent inducer of membrane process retraction by neuronal cells [24], indicating that the effects of this lipid are highly dependent on the cell type-specific coupling of LPA receptors to intracellular signaling pathways.

LPA production is associated with a variety of tissues, but the major source of LPA *in vivo* is platelets that degranulate during blood clot formation [25]. It likely that osteoblasts and osteocytes are exposed to LPA arising from the hematoma that forms following bone injury. The ability of LPA to stimulate osteocyte dendricity may help re-establish cell–cell communication in damaged bone and

foster the restoration of bone mass and mechanical integrity. Recent evidence revealed that osteoblasts can generate LPA [26], suggesting that this lipid may regulate bone cell function in situations other than skeletal repair.

Osteoblast terminal differentiation into stellate osteocytes requires cytoskeletal rearrangements [8], and we found that the osteocyte actin cytoskeleton was significantly altered in response to physiological levels of LPA. Cells exhibited enhanced stress fiber formation after 1 h of LPA treatment and, at longer time points, LPA-treated osteocytes displayed an outgrowth of elaborate dendritic membrane processes. The LPA-induced changes in stress fibers and cell morphology are similar to those seen in osteocytes subjected to oscillatory fluid flow [7], suggesting that LPA and mechanical loading may initiate dendrite outgrowth via shared signaling pathways.

LPA is a chemotactic agent for osteoblasts [15], and the current data demonstrate that MLO-Y4 osteocyte migration was elevated in response to this lipid factor. Osteocytes *in vivo* are trapped within a mineralized matrix, rendering them non-motile. However, the stimulation of MLO-Y4 cell motility suggests mechanistic links between dendrite formation and cell movement. In support of this postulate, the expression of transcripts related to cell migration were among the gene products detected in a DNA microarray analysis of calvarial osteocytes [27].

Osteocytes express transcripts encoding four LPA receptors at differential levels. LPA_1, LPA_2, and LPA_3, receptors are each coupled to $G_{i/o}$, G_q, and $G_{12/13}$; the G protein coupling status of LPA_4 is not known [20]. LPA-stimulated dendritogenesis was blocked by Ki16425, an inhibitor of LPA_1 and LPA_3 receptor forms, and by pertussis toxin. Because MLO-Y4 cells appear to express very little LPA_3 receptor mRNA, the majority of the effects of Ki16425 on dendritogenesis are likely due to an inhibition of LPA_1 function. The ability of pertussis toxin to block LPA-elicited dendrite formation demonstrates a role for $G_{i/o}$, most likely coupled to the LPA_1 receptor. Both of these agents also abrogated osteoblast migration [15], which is further evidence for the relatedness of pathways governing dendrite formation and cell motility.

LPA has a postulated role as an enhancer of mechanosensation in smooth muscle and endothelial cells [28], and we found that LPA-treated MC3T3-E1 osteoblasts exhibited distinct alterations of the actin cytoskeleton and acute elevations in Ca^{2+} [14,15], responses that also have been documented in physically stimulated osteoblasts and osteocytes [29]. Mechanical stimuli also increased osteocyte gap junction connectivity and dendrite elongation [10,30]. Thus, the stimulatory effect of LPA on osteocyte dendricity may be related to an ability of LPA to synergize with mechanotransduction in bone cells. Panupinthu et al. [26] recently reported that osteoblasts generate LPA in response to ATP that is released from these cells after mechanical stimulation, and that the released LPA was an autocrine/paracrine agonist of osteoblast membrane blebbing. These LPA-triggered osteoblast membrane extensions may relate to the osteocyte dendritogenesis we observed and further support a role for LPA in the regulation of bone cell mechanosensation.

In conclusion, we devised a simple and reproducible method to quantify osteocyte dendrite formation *in vitro* and found that LPA is a potent stimulator of osteocyte outgrowth. LPA-induced dendritogenesis required the LPA_1 receptor and coupling to $G_{i/o}$, and involved significant rearrangements of the actin cytoskeleton. Our observations of LPA-induced dendritic outgrowth and osteocyte migration provide valuable new insights into the possible mechanistic links between dendrite formation and motility. We hypothesize that LPA is a regulator of osteocyte dendrite formation *in vivo*, and that this lipid factor may have a role in re-establishing the connectivity of the osteocyte mechanosensory network during bone fracture healing.

Acknowledgments

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References

[1] M.L. Knothe Tate, J.R. Adamson, A.E. Tami, T.W. Bauer, The osteocyte, Int. J. Biochem. Cell Biol. 36 (2004) 1–8.
[2] G. Marotti, The osteocyte as a wiring transmission system, J. Musculoskelet. Neuronal Interact. 1 (2000) 133–136.
[3] C.H. Turner, A.G. Robling, R.L. Duncan, D.B. Burr, Do bone cells behave like a neuronal network? Calcif. Tissue Int. 70 (2002) 435–442.
[4] E.H. Burger, J. Klein-Nulend, Mechanotransduction in bone—role of the lacuno-canalicular network, FASEB J. 13 Suppl (1999) S101–S112.
[5] C. Palumbo, S. Palazzini, G. Marotti, Morphological study of intercellular junctions during osteocyte differentiation, Bone 11 (1990) 401–406.
[6] S. Zhao, Y.K. Zhang, S. Harris, S.S. Ahuja, L.F. Bonewald, MLO-Y4 osteocyte-like cells support osteoclast formation and activation, J. Bone Miner. Res. 17 (2002) 2068–2079.
[7] S.M. Ponik, J.W. Triplett, F.M. Pavalko, Osteoblasts and osteocytes respond differently to oscillatory and unidirectional fluid flow profiles, J. Cell. Biochem. 100 (2007) 794–807.
[8] K. Tanaka-Kamioka, H. Kamioka, H. Ris, S.S. Lim, Osteocyte shape is dependent on actin filaments and osteocyte processes are unique actin-rich projections, J. Bone Miner. Res. 13 (1998) 1555–1568.
[9] K. Holmbeck, P. Bianco, I. Pidoux, S. Inoue, R.C. Billinghurst, W. Wu, K. Chrysovergis, S. Yamada, H. Birkedal-Hansen, A.R. Poole, The metalloproteinase MT1-MMP is required for normal development and maintenance of osteocyte processes in bone, J. Cell Sci. 118 (2005) 147–156.
[10] K. Zhang, C. Barragan-Adjemian, L. Ye, S. Kotha, M. Dallas, Y. Lu, S. Zhao, M. Harris, S.E. Harris, J.Q. Feng, L.F. Bonewald, E11/gp38 selective expression in osteocytes: regulation by mechanical strain and role in dendrite elongation, Mol. Cell Biol. 26 (2006) 4539–4552.
[11] R. Dziak, B.M. Yang, B.W. Leung, S. Li, N. Marzec, J. Margarone, L. Bobek, Effects of sphingosine-1-phosphate and lysophosphatidic acid on human osteoblastic cells, Prostaglandins Leukot. Essent. Fatty Acids 68 (2003) 239–249.

[12] A. Grey, T. Banovic, D. Naot, B. Hill, K. Callon, I. Reid, J. Cornish, Lysophosphatidic acid is an osteoblast mitogen whose proliferative actions involve G_i proteins and protein kinase C, but not P42/44 mitogen-activated protein kinases, Endocrinology 142 (2001) 1098–1106.

[13] A. Grey, Q. Chen, K. Callon, X. Xu, I.R. Reid, J. Cornish, The phospholipids sphingosine-1-phosphate and lysophosphatidic acid prevent apoptosis in osteoblastic cells via a signaling pathway involving G_i proteins and phosphatidylinositol-3 kinase, Endocrinology 143 (2002) 4755–4763.

[14] J.M. Lyons, N.J. Karin, A role for G protein-coupled lysophospholipid receptors in sphingolipid-induced Ca^{2+} signaling in MC3T3-E1 osteoblastic cells, J. Bone Miner. Res. 16 (2001) 2035–2042.

[15] L.M. Masiello, J.S. Fotos, D.S. Galileo, N.J. Karin, Lysophosphatidic acid induces chemotaxis in MC3T3-E1 osteoblastic cells, Bone 39 (2006) 72–82.

[16] Y. Kato, J.J. Windle, B.A. Koop, G.R. Mundy, L.F. Bonewald, Establishment of an osteocyte-like cell line, MLO-Y4, J. Bone Miner. Res. 12 (1997) 2014–2023.

[17] M.F. Crouch, An automated fluorescence based assay of neurite formation, J. Neurosci. Methods 104 (2000) 87–91.

[18] C. Palumbo, S. Palazzini, D. Zaffe, G. Marotti, Osteocyte differentiation in the tibia of newborn rabbit: an ultrastructural study of the formation of cytoplasmic processes, Acta Anat. (Basel) 137 (1990) 350–358.

[19] T. Eichholtz, K. Jalink, I. Fahrenfort, W.H. Moolenaar, The bioactive phospholipid lysophosphatidic acid is released from activated platelets, Biochem. J. 291 (Pt 3) (1993) 677–680.

[20] I. Ishii, N. Fukushima, X. Ye, J. Chun, Lysophospholipid receptors: signaling and biology, Annu. Rev. Biochem. 73 (2004) 321–354.

[21] H. Ohta, K. Sato, N. Murata, A. Damirin, E. Malchinkhuu, J. Kon, T. Kimura, M. Tobo, Y. Yamazaki, T. Watanabe, M. Yagi, M. Sato, R. Suzuki, H. Murooka, T. Sakai, T. Nishitoba, D.S. Im, H. Nochi, K. Tamoto, H. Tomura, F. Okajima, Ki16425, a subtype-selective antagonist for EDG-family lysophosphatidic acid receptors, Mol. Pharmacol. 64 (2003) 994–1005.

[22] C. Palumbo, A three-dimensional ultrastructural study of osteoid-osteocytes in the tibia of chick embryos, Cell Tissue Res. 246 (1986) 125–131.

[23] P.A. Veno, D.P. Nicolella, P. Sivakumar, I. Kalajzic, D.W. Rowe, L.F. Bonewald, S.E. Harris, S.L. Dallas, Live imaging of osteocytes within their lacunae reveals cell body and dendrite motions, J. Bone Miner. Res. 21 (2006) S38.

[24] C.L. Sayas, J. Avila, F. Wandosell, Regulation of neuronal cytoskeleton by lysophosphatidic acid: role of GSK-3, Biochim. Biophys. Acta 1582 (2002) 144–153.

[25] F.N. van Leeuwen, B.N. Giepmans, L.A. van Meeteren, W.H. Moolenaar, Lysophosphatidic acid: mitogen and motility factor, Biochem. Soc. Trans. 31 (2003) 1209–1212.

[26] N. Panupinthu, L. Zhao, F. Possmayer, H.Z. Ke, S.M. Sims, S.J. Dixon, P2X7 nucleotide receptors mediate blebbing in osteoblasts through a pathway involving lysophosphatidic acid, J. Biol. Chem. 282 (2007) 3403–3412.

[27] S.E. Harris, W. Yang, M.A. Harris, J. Gluhak-Heinrich, L.F. Bonewald, D.W. Rowe, I. Kalajzic, Osteocyte gene expression signatures indicate that neural, muscle and cytoskeletal genes as well as wnt signaling represent novel pathways for osteocyte function, J. Bone Miner. Res. 21 (2006) S3.

[28] H. Ohata, K.I. Tanaka, N. Maeyama, T. Ikeuchi, A. Kamada, M. Yamamoto, K. Momose, Physiological and pharmacological role of lysophosphatidic acid as modulator in mechanotransduction, Jpn. J. Pharmacol. 87 (2001) 171–176.

[29] J. Rubin, C. Rubin, C.R. Jacobs, Molecular pathways mediating mechanical signaling in bone, Gene 367 (2006) 1–16.

[30] B. Cheng, S. Zhao, J. Luo, E. Sprague, L.F. Bonewald, J.X. Jiang, Expression of functional gap junctions and regulation by fluid flow in osteocyte-like MLO-Y4 cells, J. Bone Miner. Res. 16 (2001) 249–259.