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Retinoic acid promotes migration of MC3T3-E1 osteoblast-like cells via RARα signaling-mediated upregulation of profilin-1 expression

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Vitamin A is required for normal human health and metabolism. Retinoic acid (RA), a vitamin A metabolite, is critical for cell differentiation and migration. The effects of RA on remodeling of bone have been thoroughly investigated. However, its role is still under debate, especially as it relates to osteoblasts, and the relationship between RA and cell migration is relatively unknown. Using scratch assays, we previously showed that addition of RA promoted the migration of MC3T3-E1 osteoblast-like cells (OBs). In the present study, the contribution of RA receptor (RAR) subtypes to RA-mediated cell migration was investigated using pharmacological experiments. Agonists for subtype RARγ, which plays a major role in osteoblast differentiation, did not affect cell migration, while agonists for RARα promoted cell migration. To confirm the contribution of RARα, knockdown of RARα was performed and the resultant OBs exhibited reduced migration. Addition of RA upregulated mRNA expression of profilin-1 (PFN1), and overexpression of PFN1 protein promoted OB migration. These results suggest that RA promotes OB migration via RARα. Furthermore, the improvement in cell migration was mediated by RA-induced upregulation of PFN1 expression. Our findings provide new pharmacological insights into the relationship between RA and osteoblasts. (J Osaka Dent Univ 2019 ; 53 : 149-156)

Key words : Retinoic acid ; Retinoic acid receptor ; Osteoblast ; Cell migration ; Profilin-1

INTRODUCTION

Retinoic acid (RA) is a vitamin A metabolite required for growth and development of the mammalian body.[1-3] In skeletal biology studies, RA promoted bone development and antler bone regeneration.[1, 4] However, a study in humans showed that serum RA levels outside the normal range elevated the risk of hip fracture.[5] In animal studies, doses of RA or injection of vitamin A affected bone mass in a site-specific manner.[6, 7] Thus, the role of RA in bone health is dependent on cell type, cell function, and tissue. RA binds to heterodimers of retinoic acid receptors (RARs) and retinoid X receptors (RXRs).[2, 8, 9] Upon binding, RARs are translocated to the nucleus where they regulate the transcription of various target genes. This intracellular signaling is the primary biological pathway activated by all-trans-RA.[9] There are three subtypes of RAR, α, β and γ, that exhibit specific functions and different localizations in mammalian tissues and cells. The roles of these three subtypes in bone remodeling have been investigated in knockout mouse studies.[1, 10] Deficiency of RARγ is associated with trabecular bone loss through increased bone resorption. Interestingly, RARα-knockout mice showed normal bone mass and remodeling.[1] Therefore, among the RAR subtypes, RARγ is considered the main subtype for maintenance of bone mass.

The relationships among bone formation, mineralization, and RA have been extensively investigated in osteoblasts, as bone-forming cells that migrate to resorbing lacunae.[1, 11-13] Some studies have indicated that addition of RA inhibited osteoblast differentiation, while others have suggested that RA promotes differentiation and bone formation *in*

vivo.[14-16] Thus, knowledge on the effects of RA on bone formation *in vitro* remains inadequate. Consequently, the role of RA both *in vivo* and *in vitro* is still under debate. During bone remodeling, osteoblasts associate with one another by moving from their original location to sites of resorbing lacunae on the bone surface. When these cells become condensed within the primordia, they shift from a fibroblastic morphology to a cuboidal morphology and begin to secrete components of the extracellular matrix. Thus, the cells are in direct contact with not only the extracellular environment, but also the neighboring cells. This contact between cells requires cell migration, which is under the control of cytoskeletal actin dynamics.[17-19] At present, the relationship between RA and cell migration is well known. In the present study, we examined the relationship between RA and migration of MC3T3-E1 osteoblast-like cells (OBs). The effect of RA on cell migration was found to be mediated by upregulated mRNA expression of certain migration-related genes, mainly profilin-1 (PFN1).

MATERIAL AND METHODS

Cell culture

OBs (RIKEN BioResource Center, Saitama, Japan) were cultured in α-Modified Eagle's Medium (α-MEM; Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37°C under 5% CO_2 in a humidified incubator. OBs were transfected with siRNAs or expression vectors, and subsequently trypsinized and replated after 24 h. RA (Nacalai Tesque, Kyoto, Japan), BMS 195614, BMS 753, CD 2665, adapalene (Tocris Bioscience, Bristol, UK), dimethyl sulfoxide (DMSO; Wako Pure Chemical Industries, Osaka, Japan), and ethanol (EtOH; Wako Pure Chemical Industries) were added to the medium by direct pipetting. All agonists and antagonists were diluted in DMSO. In migration assays, the cultures were preincubated with antagonists for 30 min before addition of RA.

Migration assay

Scratch cell migration assays were performed as described previously.[17, 19] Briefly, OBs were plated on type I collagen-coated 3.5-cm dishes at a density of 5×10^6 cells/cm^2 in α-MEM supplemented with 1% FBS or 10% charcoal-treated FBS (GE Healthcare Japan, Tokyo, Japan). The cells were incubated for 12 h and then scratched with a 200-μl pipette tip. Images were obtained at 0 and 24 h after the scratch creation and subjected to quantitative analyses with image analysis software (All in One Microscopy; Keyence, Osaka, Japan).

Gene expression assays

Gene expression was measured as previously described.[20] Total RNA was extracted using an RNeasy Kit (Qiagen KK, Tokyo, Japan) according to the manufacturer's protocol. First-strand cDNA was produced from the total RNA with a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, San Francisco, CA, USA). Quantitative real-time PCR was performed in a Step One Real-Time PCR System (Applied Biosystems) using SYBR Green and specific forward and reverse primers. The transcript levels were normalized by the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcript level. The respective forward and reverse primer sequences were: PFN1, 5′-tcactgtcaccatgactgcc-3′ and 5′-gaggtcagtactgggaacgc-3′; RARα, 5′-gaaccggactcagatgcaca-3′ and 5′-tcctgtcggtctccacagat-3′; Nck 1, 5′-gaagtttgctggcaatccttgg-3′ and 5′-ttggcgaagattcactgtcacg-3′; GAPDH, 5'-agaaggtggtgaagcaggcat-3' and 5'-cgaaggtggaagagtgggagttg-3'.

Gene knockdown and overexpression

RARα-siRNA, PFN1-siRNA, and control-siRNA (Invitrogen) were used for knockdown experiments as previously described.[17, 21] The vector constructs expressing PFN1 were generated in the pCI mammalian expression vector (Promega KK, Tokyo, Japan). OBs were transfected with either siRNAs or expression vectors using Lipofectamine 3000 (Invitrogen) according to the manufacturer's protocols. Total RNA was extracted at 24 h after transfection. In overexpression experiments, pCI mammalian expression vector without a target gene was used as

a control.

Statistical analysis

Data are expressed as mean and standard deviation (SD) and were analyzed by Student's *t*-test or analysis of variance. Tukey's Honestly Significant Difference test was applied as a post-hoc test. The level of statistical significance was set at $p < 0.05$. All analyses were performed using SPSS software (SPSS Japan, Tokyo, Japan).

RESULTS

RA mainly promotes OB migration through RARα

Cell migration assays were performed under a reduced serum condition (1% FBS) to exclude the influences of cell proliferation and endogenous RA in FBS as much as possible. The cell migration assays confirmed that addition of RA accelerated OB migration at 24 h (Fig. 1 A). To further confirm this result, the scratch widths were quantified. The scratch width at 24 h was significantly reduced by

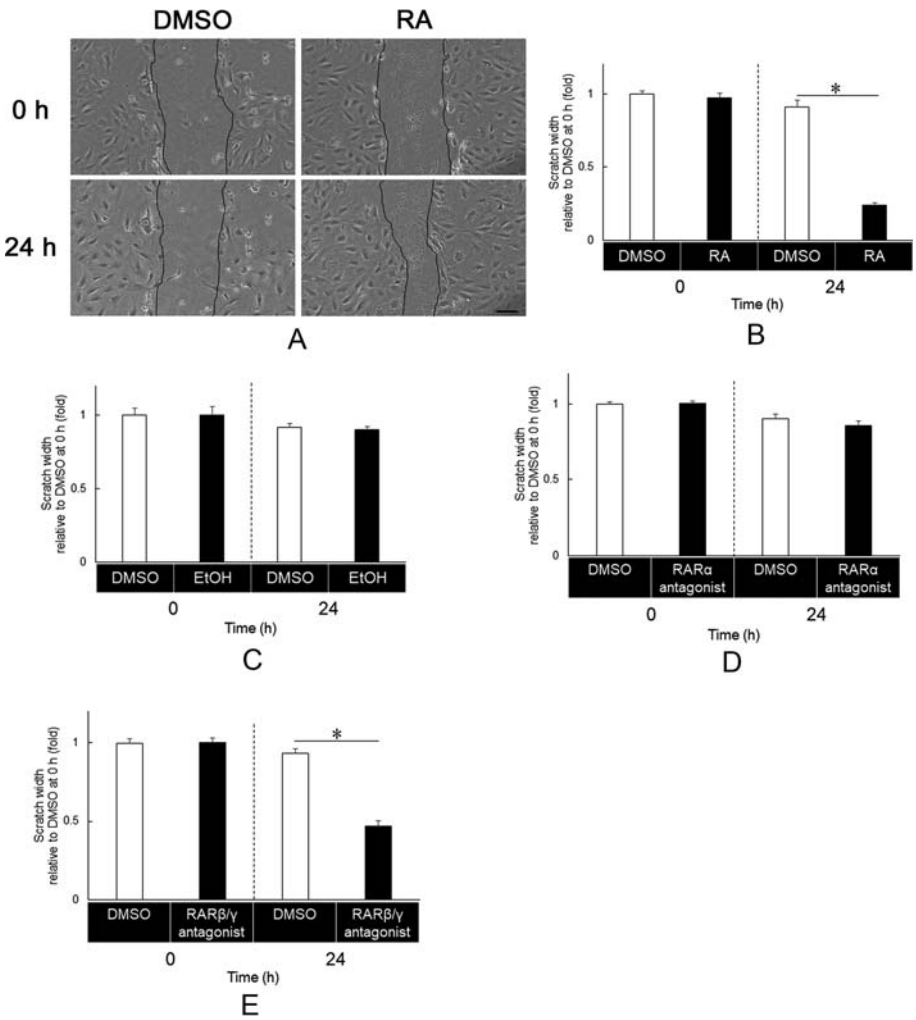

Fig. 1 RA promoting cell migration of OBs mainly via RARα. (A) Representative images of OB migration at 0 and 24 h after addition of RA (1 μM) or DMSO. (B) Scratch widths at 0 and 24 h after addition of RA (1 μM) or DMSO (Mean±SD, $n=5$, $^*p < 0.05$). (C) Scratch widths at 0 and 24 h after addition of DMSO or EtOH (Mean±SD, $n=5$). (D) Scratch widths at 0 and 24 h after addition of RA with RARα antagonist (BMS 195614 ; 1 μM) or DMSO (Mean±SD). (E) Scratch widths at 0 and 24 h after addition of RA with RARβ/γ antagonist (CD 2665 ; 0.3 μM) or DMSO (Mean±SD, $n=5$, $^*p < 0.05$).

addition of RA compared with addition of DMSO (Fig. 1 B). Thus, we concluded that RA promotes OB migration. There was no difference in the scratch widths when RA was diluted in DMSO or EtOH (Fig. 1 C). Therefore, all agonists and antagonists were diluted in DMSO to avoid the use of various solvents in the study.

Osteoblasts express the conventional RARs, including all three of the subtypes, RARα, RARβ and RARγ.[1-3] To identify the main subtype that affects RA-activated OB migration, selective antagonists were used for cell migration assays. RARα antagonist BMS 195614 inhibited RA-induced OB migration at 24 h (Fig. 1 D), while RARβ/γ antagonist CD 2665 had no effect (Fig. 1 E). These results suggest that RA mainly promotes OB migration through RARα. To investigate the side effects of FBS on RA signaling, 10% charcoal-treated FBS, containing reduced RA, was added to the cell medium instead of standard FBS. Similar to the results under the reduced serum condition, RA plus RARα antagonist, but not RA plus RARβ/γ antagonist, promoted OB cell migration in cell medium containing charcoal-treated FBS (Figs. 2 A-2 C). Thus, RA itself can affect OB cell migration.

Gene knockdown of RARα inhibits RA-induced OB migration

Gene knockdown of RARα was performed to further investigate the role of RARα in OB migration. RARα-siRNA transfection significantly reduced mRNA expression of RARα compared with control-siRNA transfection (Fig. 3 A). In RARα-knockdown (KD) cells, RA did not reduce the scratch width at 24 h after addition of RA (Fig. 3 B). Similar results were obtained in cell medium supplemented with charcoal-treated FBS (Fig. 3 C). Thus, RARα is critical for RA-induced OB migration.

Activation of RARα by RA upregulates mRNA expression of PFN1

Next, we investigated the RA signaling pathway for

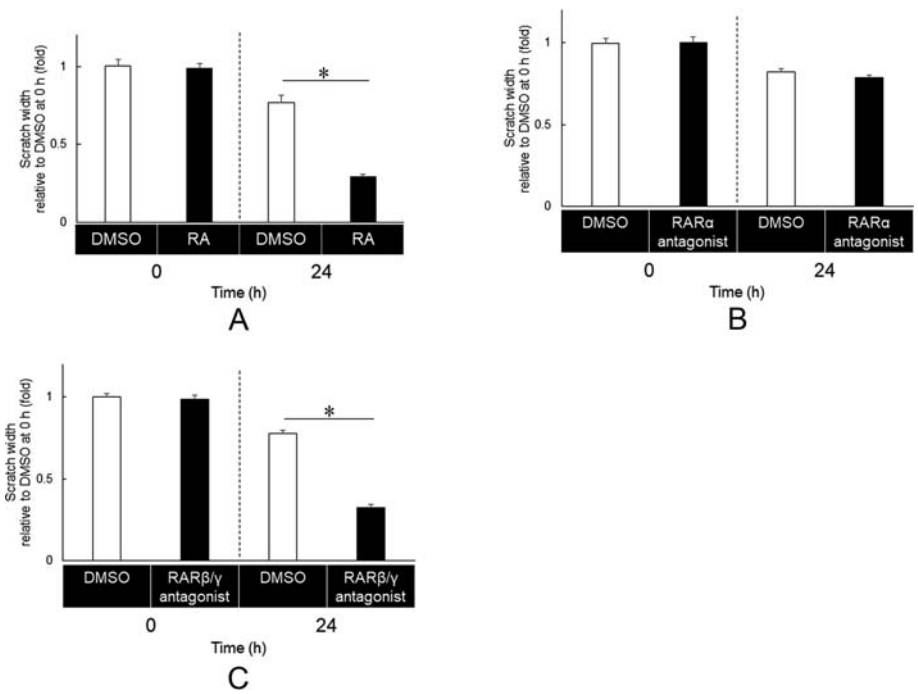

Fig. 2 RA promotion of cell migration of OBs in α-MEM supplemented with charcoal treated-FBS. (A) Scratch widths at 0 and 24 h after addition of RA (1 μM) or DMSO in α-MEM supplemented with charcoal-treated FBS (α-MEM with chFBS) (Mean±SD, $n=5$, $*p<0.05$). (B) Scratch widths at 0 and 24 h after addition of RA with RARα antagonist (BMS 195614 ; 1 μM) or DMSO in α-MEM with chFBS (Mean±SD, $n=5$). (C) Scratch widths at 0 and 24 h after addition of RA with RARβ/γ antagonist (CD 2665 ; 0.3 μM) or DMSO in α-MEM with chFBS (Mean±SD, $n=5$, $*p<0.05$).

OB migration. RA regulates the expression of several genes via translocation of RARs to the nucleus upon RA binding.[1-3] Our previous studies showed that two actin cytoskeleton-related molecules, PFN1 and Nck 1, regulate osteoblast migration.[17, 19] Thus, we focused our efforts on these genes and investigated their expression levels in RA-treated and RAR agonist-treated OBs. Addition of RA upregulated mRNA expression of PFN1, but not Nck 1 (Figs. 4 A, B). Similarly, addition of RARα agonist BMS 753 upregulated mRNA expression of PFN1 (Fig. 4 A). In contrast, RARβ/γ agonist CD 2665

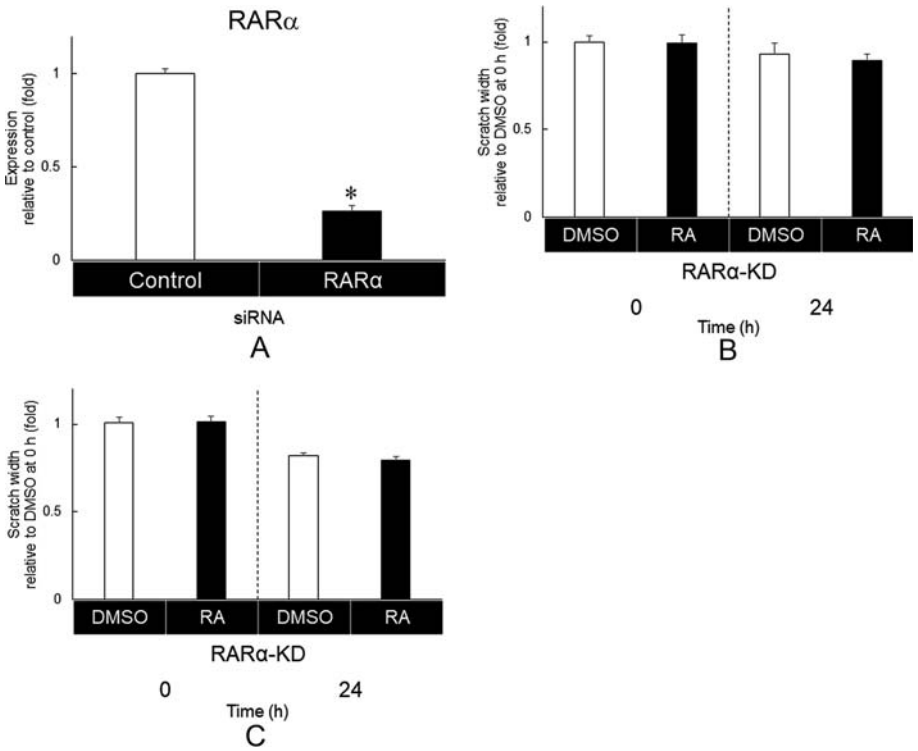

Fig. 3 RARα knockdown inhibition of RA-induced OB migration. (A) RARα knockdown by siRNA transfection, showing reduced expression of RARα at 24 h after transfection of control-siRNA or RARα-siRNA (Mean±SD, $n=5$, $*p<0.05$). (B) Scratch widths at 0 and 24 h after addition of RA (1 μM) or DMSO in RARα-KD cells (Mean±SD, $n=5$). (C) Scratch widths at 0 and 24 h after addition of RA (1 μM) or DMSO in RARα-KD cells in α-MEM with chFBS (Mean±SD, $n=5$).

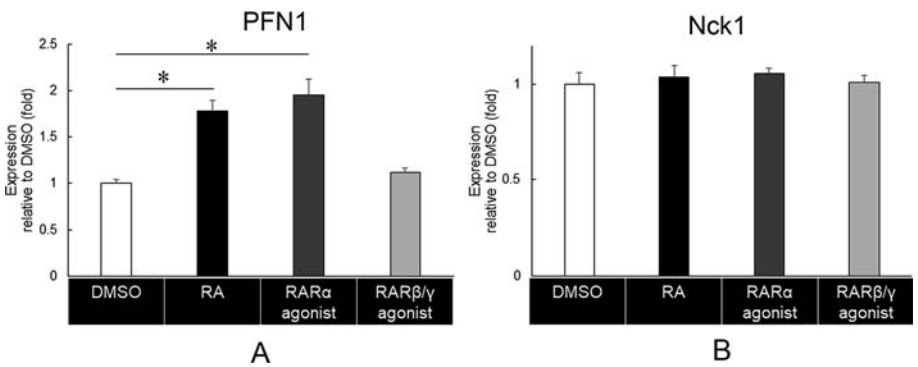

Fig. 4 Upregulation of the expression of PFN1 by the activation of RARα and the addition of RA. (A) Expression levels of PFN 1 in OBs treated with DMSO, RA, RARα agonist (BMS 753 ; 100 nM) or RARβ/γ agonist (adapalene ; 100 nM) (Mean±SD, $n=5$, $*p<0.05$). (B) Expression levels of Nck 1 in OBs treated with DMSO, RA, RARα agonist (BMS 753 ; 100 nM), or RARβ/γ agonist (adapalene ; 100 nM) in OBs (Mean±SD, $n=5$).

had no effect on mRNA expression of PFN1. Neither RARα agonist nor RARβ/γ agonist affected mRNA expression of Nck 1 (Fig. 4 B). These results suggest that RA upregulates PFN1 mRNA expression via RARα signaling.

Overexpression of PFN1 improves OB migration

In our previous study, knockdown of PFN1 mRNA inhibited migration in primary osteoblasts.[17] We performed overexpression experiments to further confirm the role of PFN1 in cell migration. PFN1 mRNA expression was increased compared with the control at 24 h after transfection (Fig. 5 A). In PFN1-overexpressing OBs, the scratch width was reduced compared with that in OBs after control vector transfection (Fig. 5 B). In addition to the overexpression experiments, PFN1 mRNA was knocked down by PFN1-siRNA transfection. The mRNA expression of PFN1 was reduced compared with the control at 24 h after PFN1-siRNA transfection (Fig.

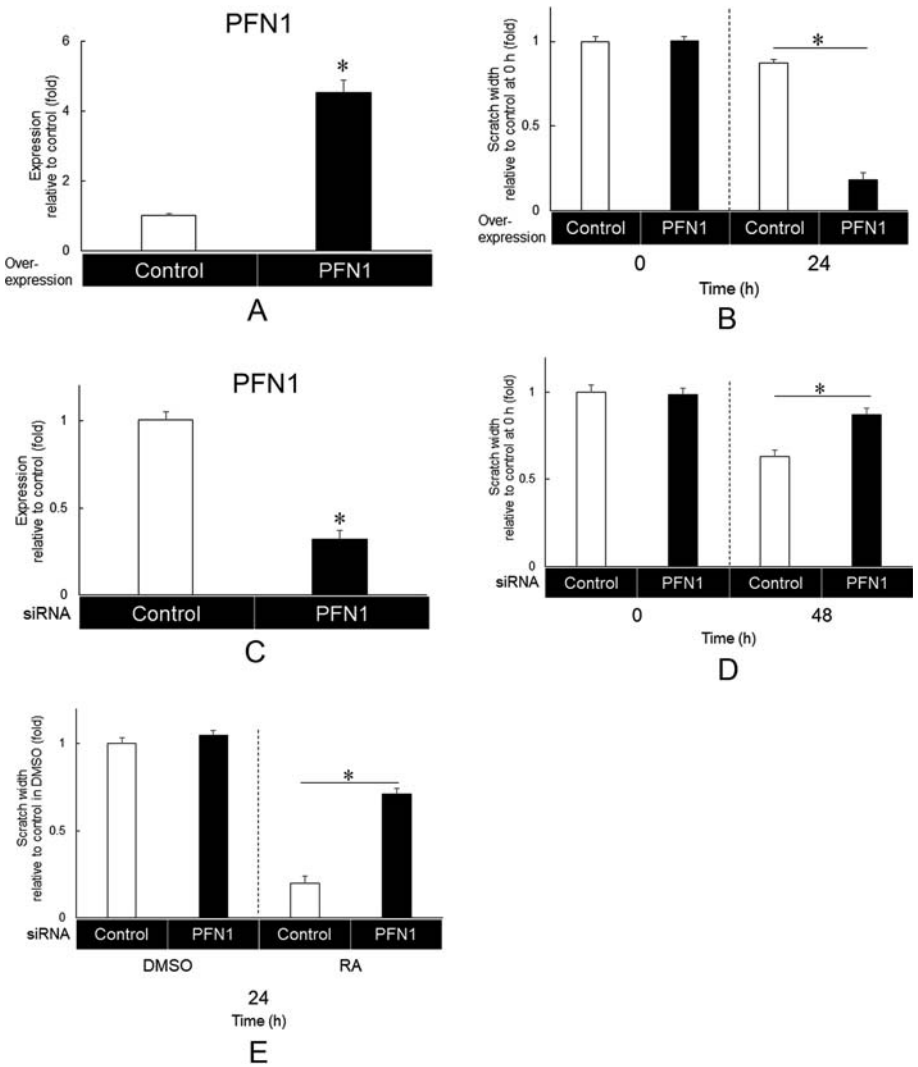

Fig. 5 Promotion of OB migration by increased expression of PFN1. (A) Increased expression of PFN1 at 24 h after transfection with control vector or PFN1 expression vector (Mean±SD, $n=5$, $*p<0.05$). (B) Scratch widths at 0 and 24 h in control or PFN1-overexpressing OBs without addition of RA (Mean±SD, $n=5$, $*p<0.05$). (C) Reduction of expression of PFN1 at 24 h after transfection of control-siRNA or PFN1-siRNA (Mean±SD, $n=3$, $*p<0.05$). (D) Scratch widths at 0 and 48 h in control or PFN1-knockdown OBs without addition of RA (Mean±SD, $n=5$, $*p<0.05$). (E) Scratch widths at 24 h in control or PFN1-knockdown OBs with or without addition of RA (Mean±SD, $n=5$, $*p<0.05$).

5 C). The reduced PFN1 mRNA expression inhibited OB migration (Fig. 5 D). Addition of RA promoted OB migration, and the effects were reduced in PFN1-knockdown OBs (Fig. 5 E). These data suggest that an increase in PFN1 mRNA expression improves OB migration.

DISCUSSION

The present study demonstrates that RA promotes cell migration through upregulation of PFN1 expression via RARα in OBs. The effects of RA on various cellular responses of osteoblasts have been extensively investigated.[2, 14-16] However, even though the role of RA in osteoblast differentiation has been investigated by many research groups,[1, 11-13] its effect on OB migration is not well established. The present results suggest a positive role of RA in bone metabolism.

RA signaling is one of most important mechanisms for bone metabolism. As an example for bone regeneration, RA is critical in deer antler remodeling.[4] Specifically, RA is present in the growing antler and regulates the differentiation of chondrocytes, osteoblasts, and osteoclasts *in vivo* and *in vitro.* During antler regeneration, these cells migrate to sites of regeneration and activate bone remodeling. The present study showed that RA-induced cell signaling improved OB migration. Therefore, in bone regeneration, RA will affect not only differentiation of osteoblasts, but also cell migration. For bone fracture healing, improvement of osteoblast functions and gathering of cells to the fracture point both have therapeutic effects.[22, 23] Our findings may be of help in further understanding the mechanisms of bone regeneration and fracture healing.

PFN1 is an actin monomer-binding protein that has been implicated in various cellular functions, including cell migration.[24] In our previous study, PFN1 deficiency suppressed osteoblast migration and reduced stress fibers composed of actin filaments.[17] The present data support these functions of PFN1. Both RA-induced upregulation of PFN1 and overexpression of PFN1 were related to improved OB migration, while knockdown of PFN1 reduced the effects of RA on OB migration. Although RA can regulate the expression of many genes,[1-3] PFN1 is one of the factors through which RA promotes cell migration.

RA regulates the expression of its target genes by binding to heterodimers of RARs and RXRs. RA can also activate other nuclear receptors, such as peroxisome proliferator-activated receptor β/γ (PPARβ/γ). Although PPARs can bind to RA, their affinities for RA are weaker than those of RARs.[25] Although the effects of PPARγ on RA-induced cell migration were not examined in this study, PPARγ is expressed in bone and its activation inhibits osteoblast differentiation.[26, 27] Based on the finding that RARα knockdown inhibited RA-induced cell migration, the effects of PPARγ on osteoblast migration are deemed to be low. The relationship between RA and PPARγ will be further assessed in future studies.

It is noteworthy that a reduced serum condition (1% FBS) was used for the cell migration assays in the present study. Under standard serum condition (10% FBS), the scratch widths showed no significant difference between addition of DMSO and RA (data not shown). The reason could be the high amount of endogenous RA in FBS. The data from assays with charcoal-treated FBS (Figs. 2 A-C) also support this speculation. Saturation of RARs by the RA present in 10% FBS may yield no discernable difference in scratch widths regardless of whether external RA is added. Therefore, RA should be used under limited conditions for improvement of OB migration.

In summary, our results demonstrate a role for RA in osteoblast migration. RA regulated osteoblast migration via RARα signaling and subsequent upregulation of PFN1 mRNA expression. Overexpression of PFN1 promoted cell migration. The present findings improve our understanding of bone biology and may be useful tools for pharmacological and therapeutic regulation of cell migration in bone.

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REFERENCES

1. Green AC, Martin TJ, Purton LE. The role of vitamin A and retinoic acid receptor signaling in post-natal maintenance of bone. *J Steroid Biochem Mol Biol* 2016 ; **155** : 135-146.
2. Conaway HH, Henning P, Lerner UH. Vitamin A metabolism, action, and role in skeletal homeostasis. *Endocr Rev* 2013 ; **34** : 766-797.
3. Henning P, Conaway HH, Lerner UH. Retinoid receptors in bone and their role in bone remodeling. *Front Endocrinol (Lausanne)* 2015 ; **6** : 31.
4. Price J, Allen S. Exploring the mechanisms regulating regeneration of deer antlers. *Philos Trans R Soc B Biol Sci* 2004 ; **359** : 809-822.
5. Wu A-M, Huang C-Q, Lin Z-K, Tian N-F, Ni W-F, Wang X-Y, Xu H-Z, Chi Y-L. The relationship between vitamin A and risk of fracture : meta-analysis of prospective studies. *J Bone Miner Res* 2014 ; **29** : 2032-2039.
6. Hotchkiss CE, Latendresse J, Ferguson SA. Oral treatment with retinoic acid decreases bone mass in rats. *Comp Med* 2006 ; **56** : 502-11.
7. Kneissel M, Studer A, Cortesi R, Susa M. Retinoid-induced bone thinning is caused by subperiosteal osteoclast activity in adult rodents. *Bone* 2005 ; **36** : 202-14.
8. Mic FA, Molotkov A, Benbrook DM, Duester G. Retinoid activation of retinoic acid receptor but not retinoid X receptor is sufficient to rescue lethal defect in retinoic acid synthesis. *Proc Natl Acad Sci USA* 2003 ; **100** : 7135-7140.
9. Al Tanoury Z, Piskunov A, Rochette-Egly C. Vitamin A and retinoid signaling : genomic and nongenomic effects. *J Lipid Res* 2013 ; **54** : 1761-1775.
10. Lohnes D, Kastner P, Dierich A, Mark M, LeMeur M, Chambon P. Function of retinoic acid receptor gamma in the mouse. *Cell* 1993 ; **73** : 643-58.
11. Lind T, Sundqvist A, Hu L, Pejler G, Andersson G, Jacobson A, Melhus H. Vitamin A is a negative regulator of osteoblast mineralization. *PLoS One* 2013 ; **8** : e 82388.
12. Togari A, Kondo M, Arai M, Matsumoto S. Effects of retinoic acid on bone formation and resorption in cultured mouse calvaria. *Gen Pharmacol* 1991 ; **22** : 287-92.
13. Ohishi K, Nishikawa S, Nagata T, Yamauchi N, Shinohara H, Kido J, Ishida H. Physiological concentrations of retinoic acid suppress the osteoblastic differentiation of fetal rat calvaria cells *in vitro*. *Eur J Endocrinol* 1995 ; **133** : 335-41.
14. Dingwall M, Marchildon F, Gunanayagam A, Louis CS, Wiper-Bergeron N. Retinoic acid-induced Smad 3 expression is required for the induction of osteoblastogenesis of mesenchymal stem cells. *Differentiation* 2011 ; **82** : 57-65.
15. Gazit D, Ebner R, Kahn AJ, Derynck R. Modulation of expression and cell surface binding of members of the transforming growth factor-beta superfamily during retinoic acid-induced osteoblastic differentiation of multipotential mesenchymal cells. *Mol Endocrinol* 1993 ; **7** : 189-98.
16. Hisada K, Hata K, Ichida F, Matsubara T, Orimo H, Nakano T, Yatani H, Nishimura R, Yoneda T. Retinoic acid regulates commitment of undifferentiated mesenchymal stem cells into osteoblasts and adipocytes. *J Bone Miner Metab* 2013 ; **31** : 53-63.
17. Miyajima D, Hayata T, Suzuki T, Hemmi H, Nakamoto T, Notomi T, Amagasa T, Böttcher RT, Costell M, Fässler R, Ezura Y, Noda M. Profilin1 regulates sternum development and endochondral bone formation. *J Biol Chem* 2012 ; **287** : 33545-33553.
18. Olson EN, Nordheim A. Linking actin dynamics and gene transcription to drive cellular motile functions. *Nat Rev Mol Cell Biol* 2010 ; **11** : 353-365.
19. Aryal A C S, Miyai K, Izu Y, Hayata T, Notomi T, Noda M, Ezura Y. Nck influences preosteoblastic/osteoblastic migration and bone mass. *Proc Natl Acad Sci USA* 2015 ; **112** : 15432-7.
20. Notomi T, Kuno M, Hiyama A, Ezura Y, Honma M, Ishizuka T, Ohura K, Yawo H, Noda M. Membrane depolarization regulates intracellular RANKL transport in non-excitable osteoblasts. *Bone* 2015 ; **81** : 306-14.
21. Notomi T, Kuno M, Hiyama A, Ohura K, Noda M, Skerry TM. Zinc-induced effects on osteoclastogenesis involves activation of hyperpolarization-activated cyclic nucleotide modulated channels via changes in membrane potential. *J Bone Miner Res* 2015 ; **30** : 1618-1626.
22. Su P, Tian Y, Yang C, Ma X, Wang X, Pei J, Qian A. Mesenchymal stem cell migration during bone formation and bone diseases therapy. *Int J Mol Sci* 2018 ; **19** : 2343.
23. Wasnik S, Rundle CH, Baylink DJ, Yazdi MS, Carreon EE, Xu Y, Qin X, Lau K-HW, Tang X. 1,25-Dihydroxyvitamin D suppresses M 1 macrophages and promotes M2 differentiation at bone injury sites. *JCI Insight* 2018 ; **3** : e98773. doi. org/10.1172/jci.insight.98773.
24. Witke W. The role of profilin complexes in cell motility and other cellular processes. *Trends Cell Biol* 2004 ; **14** : 461-469.
25. Xu HE, Lambert MH, Montana VG, Parks DJ, Blanchard SG, Brown PJ, Sternbach DD, Lehmann JM, Wisely GB, Willson TM, Kliewer SA, Milburn M V. Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. *Mol Cell* 1999 ; **3** : 397-403.
26. Jeon MJ, Kim JA, Kwon SH, Kim SW, Park KS, Park S-W, Kim SY, Shin CS. Activation of peroxisome proliferator-activated receptor-gamma inhibits the Runx 2-mediated transcription of osteocalcin in osteoblasts. *J Biol Chem* 2003 ; **278** : 23270-7.
27. Stechschulte LA, Czernik PJ, Rotter ZC, Tausif FN, Corzo CA, Marciano DP, Asteian A, Zheng J, Bruning JB, Kamenecka TM, Rosen CJ, Griffin PR, Lecka-Czernik B. PPARG post-translational modifications regulate bone formation and bone resorption. *EBioMedicine* 2016 ; **10** : 174-84.