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ORIGINAL ARTICLE

Pigment epithelium-derived factor (PEDF) reduced expression and synthesis of SOST/sclerostin in bone explant cultures: implication of PEDF-osteocyte gene regulation in vivo

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Abstract
Mutations in Serpinf1 gene which encodes pigment epithelium-derived factor (PEDF) lead to osteogenesis imperfecta type VI whose hallmark is defective matrix mineralization. We reported previously that PEDF reduced expression and synthesis of Sost/Sclerostin as well as other osteocytes genes encoding proteins that regulate matrix mineralization [1]. To determine whether PEDF had an effect on osteocyte gene expression in bone, we used bone explant cultures. First, osteocytes were isolated from surgical waste of bone fragments obtained from patients undergoing elective foot surgeries under approved IRB protocol by Penn State College of Medicine IRB committee. Primary osteocytes treated with PEDF reduced expression and synthesis of Sost/Sclerostin and matrix phosphoglycoprotein (MEPE) as well as dentin matrix protein (DMP-1). On the whole, PEDF reduced osteocyte protein synthesis by 50% and by 75% on mRNA levels. For bone explants, following collagenase digestion, bone fragments were incubated in alpha-MEM supplemented with 250 ng/ml of PEDF or BSA. After 7 days of incubation in a medium supplemented with PEDF, analysis of mRNA by PCR and protein by western blotting of encoded osteocyte proteins showed reduced Sclerostin synthesis by 39% and MEPE by 27% when compared to fragments incubated in medium supplemented with BSA. mRNA expression levels of osteocytes in bone fragments treated with PEDF were reduced by 50% for both SOST and MEPE when compared to BSA-treated bone fragments. Taken together, the data indicate that PEDF has an effect on osteocyte gene expression in bone and encourage further studies to examine effect of PEDF on bone formation indices in animal models and its effect on osteocyte gene expression in vivo following PEDF administration.

Keywords Pigment epithelium derived factor · Sclerostin · Osteocytes · Bone explant culture · Osteogenesis imperfecta

Introduction

Pigment epithelium-derived factor (PEDF) encoded by serpinf1 was originally identified in cultures of fetal pigment epithelial cells but is now known to be ubiquitously synthesized by a variety of cell types including osteoblasts and osteocytes [2–5]. PEDF has been shown to possess a strong antiangiogenic activity and this property has heightened interest in understanding its potential to ameliorate metastasis of some cancers and effects of diabetes like retinopathy [2, 6, 7]. The importance of PEDF in bone was revealed by the discovery that patients with a rare recessive form of osteogenesis imperfecta type VI carry mutations in serpinf1 gene, a gene encoding PEDF. Serpinf1 gene mutations lead to excessive accumulation of osteoid in bone that fails to mineralize [8–10]. Mechanisms, by which PEDF regulates bone matrix mineralization, remain undefined. We and

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others reported that PEDF promoted human mesenchymal stem cell (MSC) differentiation and increased osteoblast mineralization [11–13]. We also reported that PEDF suppressed Sost/Sclerostin expression by primary osteocytes harvested from human bone [1]. In addition to Sost/Sclerostin suppression, we also reported that PEDF had an effect on the expression of other osteocyte genes, MEPE, DMP-1 and PHEX [1]. PEDF inhibited expression of SOST/Sclerostin, MEPE and to a lesser extent DMP-1 [1]. These osteocyte proteins play major roles in matrix mineralization and thus, PEDF may play a role in matrix mineralization by regulating genes involved in bone matrix mineralization [1].

Osteocytes are notoriously known to be difficult to isolate and maintain in culture and thus, various techniques have been developed to determine osteocyte activities in environments that mimic their natural habitat. To determine whether PEDF has an effect on osteocyte gene expression in bone, we developed a bone explant culture to assess the effect of PEDF on osteocyte gene expression in bone.

Materials and methods

Isolation of primary osteocytes

For isolation of primary osteocytes, methods that we described previously and established protocols were used [1, 14–16]. Briefly, foot bones were obtained from patients undergoing elective foot surgery under approved IRB protocol (protocol # 432612EM) by the Penn State College of Medicine. Fifteen bone fragments from patients ranging from 50- to 60-year old were extensively washed in PBS followed by treatment with 5 mM EDTA in PBS containing 0.1% BSA at 37 °C for 45 min; this was followed by incubation in 0.2% type II collagenase in PBS for 45 min at 37 °C. The process was repeated two more times followed by centrifugation at 200 *g* for 5 min. Cell pellets were suspended in a medium (alpha-MEM supplemented with 10% FBS and 1% P/S) and seeded onto collagen-coated six-well plates. Cells in six-well plates were treated with PEDF at 50 ng/ml or/and 250 ng/ml, and assessed for SOST, DMP-1 and MEPE expression by RT-PCR and western blotting essentially as we described previously [1].

Ex vivo explant bone culture

To determine whether PEDF suppressed expression of Sost and other osteocyte-related genes in bone, ex vivo explant bone culture was employed using a modified method based on the previous reports examining osteocytes in bone [17–20]. Bone fragments from 12 patients aged 50–60 years who were undergoing elective foot surgeries were cut into 4×4×2 mm cubes and washed in PBS. Following washing, each bone chip was placed into a conical tube and incubated in 2 ml of 0.2% (w/v) collagenase type II in a 37 °C water bath with shaking for 45 min. After 45 min of incubation, each bone chip was washed with PBS twice and placed into a 24-well plate, incubated in alpha-MEM supplemented with 10% FBS, 1% P/S for 24 h. Bone chips were maintained in the medium in the presence or absence of 250 ng/ml of PEDF. Control bone chips were incubated in a medium supplemented with BSA. Bone chips were maintained in the medium either supplemented with PEDF or BSA which was replaced every day for 7 days. After 7 days of incubation, bone chips were collected and washed in PBS twice followed by extraction of mRNA or proteins within. For protein extraction, the chips were suspended in 500 µl lysis buffer (20 mM Tris–HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM $Na_4P_2O_7$, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, and 1 µg/ml leupeptin) with grinding for 5 min. The lysates were cleared by centrifugation at 20,000 *g* for 5 min. The supernatants were transferred into new tubes and protein concentrations were determined using the BCA method. Total lysates (30–60 µg protein/lane) were subjected to electrophoresis in 4–20% SDS-PAGE for western blotting. Some bone chips were treated with 1 ml of Trizol solution for mRNA isolation.

Effect of PEDF on osteocyte gene expression by primary osteocytes

Quantitative real-time PCR

Quantitative PCR was carried out to determine the expression of osteocyte genes, SOST, dentin matrix protein (DMP-1) and matrix extracellular phosphoglycoprotein (MEPE) by primary osteocytes or osteocytes in explant cultures. The cells were cultured in the presence or absence of 50 or 250 ng/ml of PEDF prior to mRNA analysis. mRNA expression level was analyzed in a StepOnePlus™ RT PCR system (Applied Biosystems, CA). The primers and programs used for amplification of the genes were previously reported [1].

Western blotting

Total protein was harvested from primary osteocytes following treatment with or without 50 or 250 ng/ml of PEDF for 3 days. Cells were lysed in RIPA buffer supplemented with proteinase inhibitor cocktail (Sigma–Aldrich). The protein concentration was determined with a BCA protein assay kit (Sigma–Aldrich), and 10 µg of proteins from each sample was resolved by SDS-PAGE and transferred onto PVDF

membranes. Antibodies specific for Sclerostin (Abcam, ab63097),MEPE (Santa Cruz, SC-377035) and DMP-1 (Santa Cruz, SC-73633) were used to detect expression of osteocyte proteins of interest.

Statistical analysis

For all experiments, data were compiled from at least three independent replicate experiments performed on separate cultures on separate occasions. Results were expressed as the mean ± SD. Comparative studies of means were performed using one-way ANOVA followed by a post hoc test (projected least significant difference, Fisher) with a significant value of $p < 0.05$. Expression level was analyzed in a StepOnePlus™ RT PCR system (Applied Biosystems, CA).

Results

PEDF reduced expression of Sost/Sclerostin, MEPE and DMP-1 by primary osteocytes in culture

We first examined the effect of PEDF on suppressing expression of Sost/Sclerostin by primary osteocytes examining the effect of PEDF dose. In our previous report, we did not examine the effect of PEDF dose on osteocyte gene and protein expression [1]. The data showed that PEDF reduced

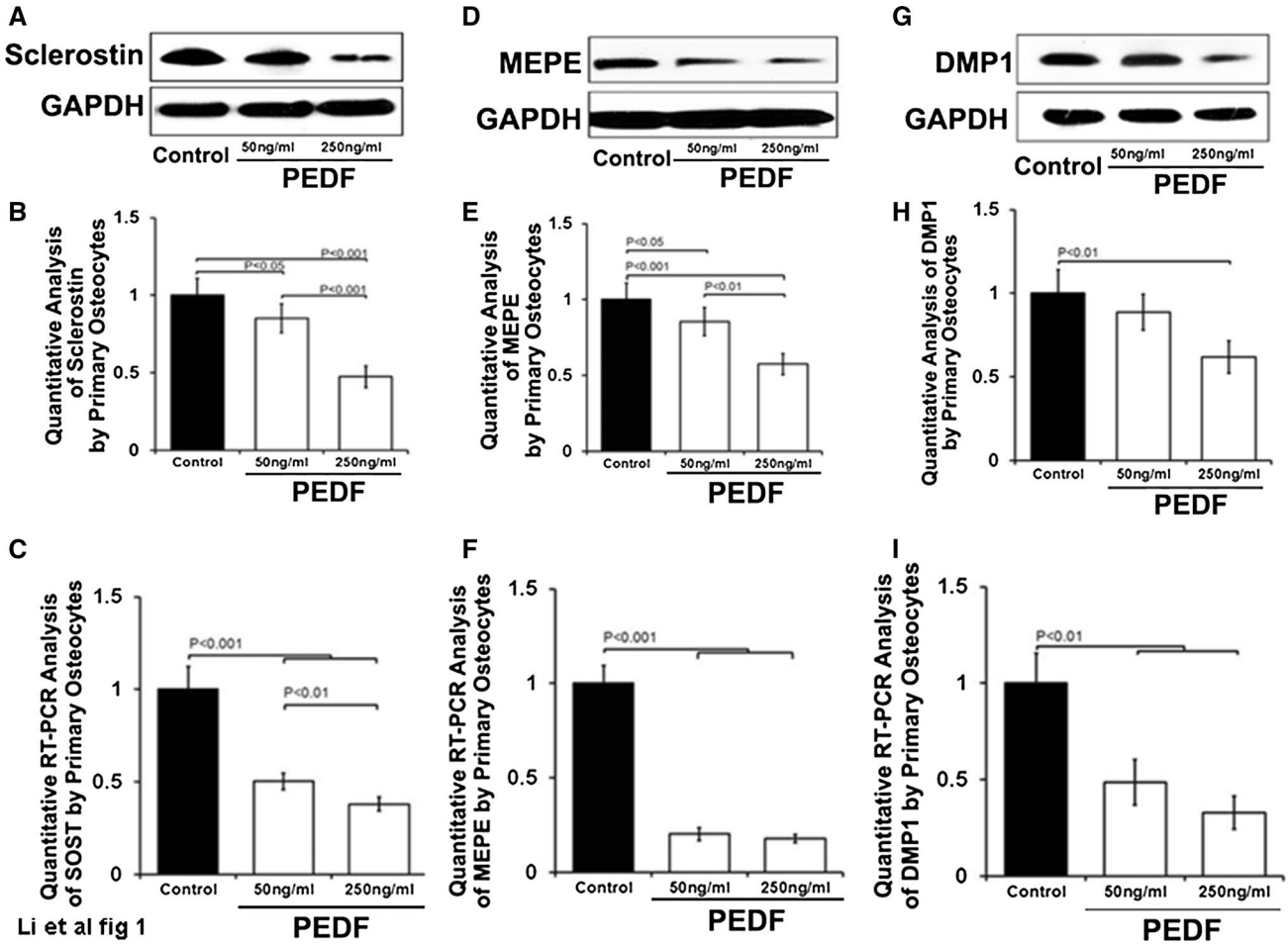

Fig. 1 PEDF reduced expression of Sclerostin, MEPE and DMP-1 proteins in human primary osteocytes. **a**, **b**, **c** Western blot and quantitative analysis of Sclerostin synthesis by primary osteocytes, and mRNA levels in osteocytes cultured in a medium supplemented with two different doses of PEDF. PEDF suppressed SOST/Sclerostin expression by osteocytes in a dose-dependent manner. **d**, **e**, **f** Western blot and quantitative analysis of MEPE synthesis, and mRNA levels by primary osteocytes cultured in a medium supplemented with two different doses of PEDF. PEDF suppressed MEPE synthesis in a dose-dependent manner but both 50 ng and 250 ng/ml of PEDF reduced expression of MEPE equally well. **g**, **h**, **i** Western blot and quantitative analysis of DMP-1 synthesis, and mRNA expression levels by osteocytes in a medium supplemented with PEDF; DMP-1 synthesis and mRNA expression were reduced upon PEDF treatment. In all the cases, 50 ng and 250 ng/ml of PEDF were effective in suppressing synthesis and expression of DMP-1. All samples were treated with/without PEDF for 72 h; $N = 4$ and each experiment was performed in triplicates. Data are presented as mean ± SD

expression and synthesis of Sclerostin, MEPE and DMP-1 by primary osteocytes as shown by western blotting (Fig. 1a, b for Scl, Fig. 1d, e for MEPE and Fig. 1g, h for DMP-1). mRNA expression levels were also assessed by RT-PCR following PEDF treatment (Fig. 1c, f, i). Sost expression was reduced by PEDF in a dose-dependent manner (Fig. 1b, c) while for MEPE reduction dose dependency was only observed on its synthesis (Fig. 1d, e). For DMP-1, both PEDF doses were equally efficient in suppressing its expression and synthesis (Fig. 1g, h, i). Control osteocytes were cultured in a medium without PEDF supplement. Dose dependence of mRNA reduction by osteocytes upon PEDF treatment was seen for SOST expression while for MEPE and DMP-1 both doses of PEDF were equally effective (Fig. 1c, f, i). These findings go along with what we reported previously, but in this report, we examined the effect of PEDF dose on Sclerostin, MEPE and DMP-1 expression. In addition, the data support a recent report in which we showed that long-term osteoblast culture contained osteocytes that responded to PEDF to regulate osteocyte gene expression [21].

PEDF suppressed synthesis and expression of Sclerostin/Sost and MEPE by osteocytes in bone

We next examined whether PEDF had an effect on osteocytes within bone tissue by examining the effect of PEDF on osteocyte gene expression and protein synthesis in bone explants. Western blotting and PCR were used to determine the effect of PEDF on SOST/Scl, MEPE mRNA and protein levels. Western blot analysis of the proteins extracted from bone fragments incubated in PEDF showed that 7 of the 12 bone fragments treated with PEDF synthesized less Sclerostin when compared to bone fragments not treated with PEDF as shown by western blot and quantitative analysis (Fig. 2a– c). On an average, PEDF reduced 39% of Sclerostin synthesis by osteocytes in bone fragments. Similarly, bone fragments incubated in PEDF for 7 days showed reduced MEPE synthesis as shown by western blotting and quantitation of the blots (Fig. 2a, d, e). Reduction in MEPE protein synthesis by PEDF was about 27%; this is relatively modest when compared to reduction in Sclerostin synthesis. Because PEDF was exogenously added, it is not clear of the amount that was accessible to osteocytes in bone. The data nevertheless suggest that PEDF has an effect on osteocyte protein synthesis in bone.

In addition to examining the effect of PEDF on protein synthesis by PEDF, we also assessed its effect on SOST and MEPE gene expression by osteocytes in bone. Bone fragments treated with or without PEDF were powdered and mRNA therein was extracted. Analysis of the mRNA levels revealed that bone fragments treated with PEDF expressed reduced mRNA levels for SOST (Fig. 3a) and MEPE (Fig. 3b). Overall, mRNA for SOST and MEPE expression by osteocytes in bone was reduced by 50% upon PEDF treatment. Interestingly, human PEDF had no effect on osteocyte gene expression by mouse bones (data not shown). Use of BSA as a control was appropriate because cells treated with BSA showed stability in terms of GAPDH expression (Fig. 1 supplement).

Discussion

PEDF is a multifunctional protein whose absence has been shown to lead to the development of OI type VI, thus, it plays an important role in bone; mechanisms of its action, however, remain undefined [8–10]. The hallmark of type VI OI is osteoid buildup that fails to mineralize [8–10]. The role of PEDF in bone mineralization is only a matter of speculation [1, 9, 13]. We and others reported that PEDF enhanced osteoblast differentiation and matrix mineralization by enhancing the expression of osteoblast-related genes [1, 11–13]. We also reported that PEDF suppressed expression of osteocyte-related genes including Sost [1]. We speculated that suppression of osteocyte-related genes that play a role in regulating matrix mineralization may be part of the mechanisms by which PEDF regulates matrix mineralization. Indeed, several reports have shown that Sclerostin plays a role in bone mineralization [22–26]. In the present report, we have shown that in addition to Sclerostin inhibition, PEDF also reduced expression of MEPE and DMP-1, and these osteocyte proteins have been shown to play a role in bone mineralization [27–31].

We have shown that PEDF regulates Sclerostin in a dose-dependent manner. Our previous report on Sost/Sclerostin suppression by PEDF was based on studies carried out in primary osteocytes [1]; in the present report, we have extended the studies to examine osteocytes in bone. We used ex vivo explant bone culture to examine the effect of PEDF on regulating osteocyte gene expression and synthesis of encoded proteins. This approach has been used previously by other investigators examining various aspects of bone biology [17–20, 32]. Using this system, we have shown that PEDF regulates osteocytes expression in bone; the present findings provide an opportunity to test the effect of PEDF in suppressing Sost/Scl expression in bone using animal models. Because PEDF is a large molecule, bone fragments were first digested with collagenase to allow PEDF access to the osteocytes. There was

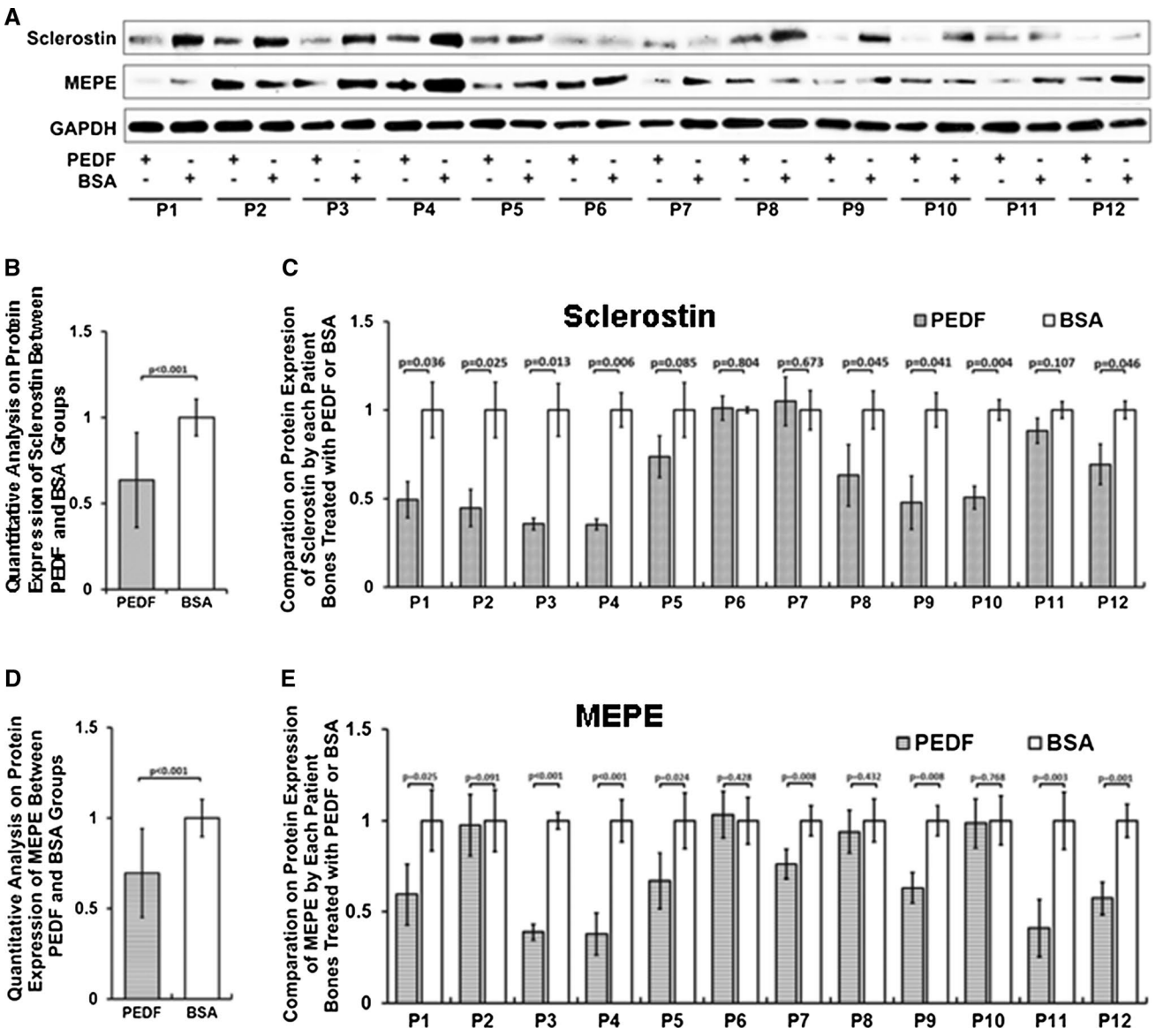

Fig. 2 PEDF reduced expression of Sclerostin and MEPE by human osteocytes in human bone fragments in ex vivo explant culture. **a** Western blot of Sclerostin and MEPE synthesis by osteocytes in bone explants reveal suppression of their synthesis by treatment with PEDF. **b**, **c** Quantitation of the western blot for Sclerostin synthesis in bone explants treated with PEDF showing its reduced synthesis. **d**, **e** Quantitation of the western blot shown in **a** for MEPE synthesis in bone explants treated with PEDF showing reduced synthesis of MEPE. Bone fragments were incubated in DMEM supplemented with or without 250 ng/ml of PEDF for 7 days. Proteins were extracted thereafter and subjected to gel electrophoresis and subsequent western blotting. Experiments were done in triplicates. Data are presented as mean ± SD

variability of osteocyte in different bone fragment response to PEDF; the reason is not clear but it could result from failure of exogenous PEDF to access osteocytes because of the presence of interfering matrix or osteocytes in bone of different patients which may respond differently. These speculations are beyond the studies reported in the present manuscript. PEDF was supplied exogenously; it is likely that osteocytes may respond to PEDF via autocrine

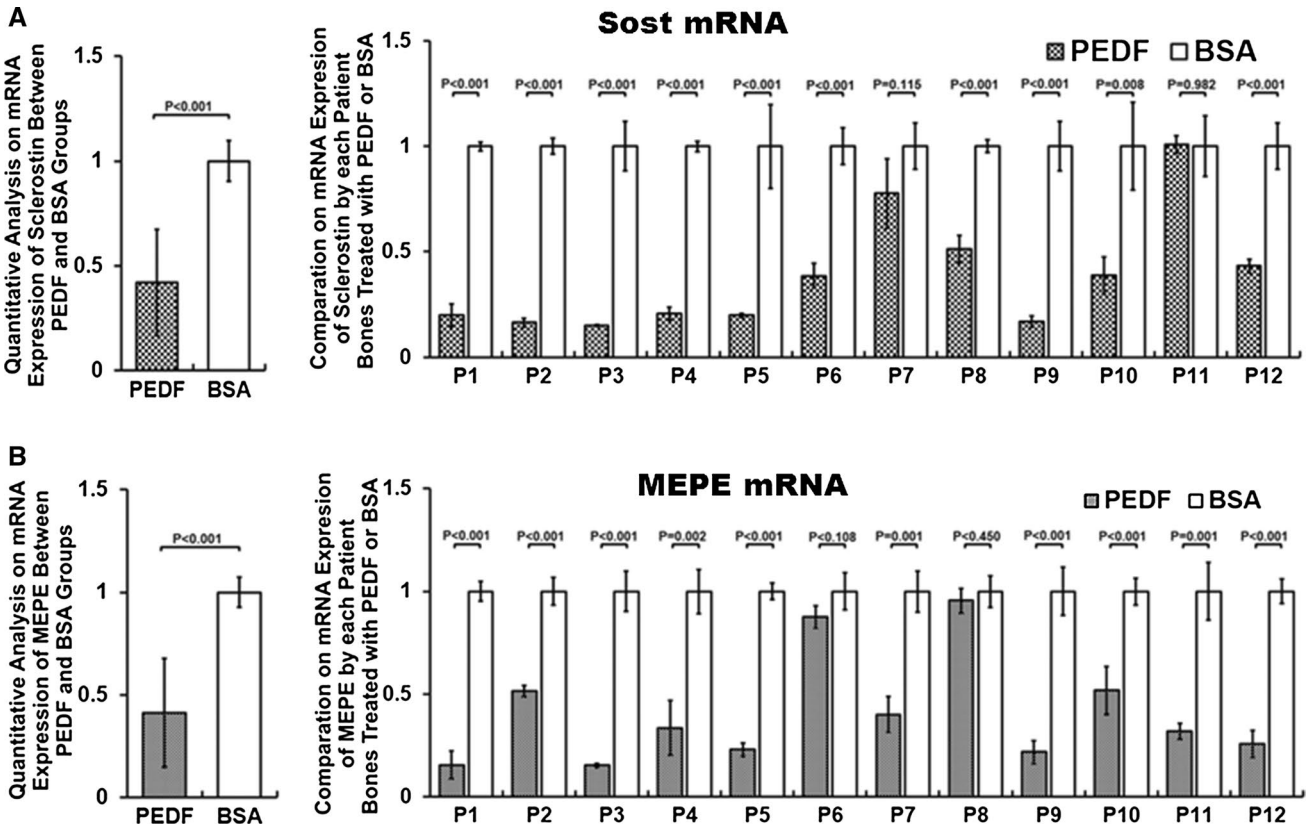

Fig. 3 PEDF reduced mRNA expression of SOST and MEPE in human bone explant cultures. **a** Quantitative PCR analysis of mRNA levels revealed suppression of SOST in bone explants incubated in PEDF. **b** Quantitative PCR analysis of mRNA revealed suppression of MEPE expression by PEDF in bone explants incubated with PEDF. Human bone fragments were incubated in a medium supplemented with 250 ng/ml of PEDF for 7 days. Control bone fragments were incubated in a medium supplemented with BSA. Data are presented as mean ± SD

mechanism since osteocytes produce PEDF thus, PEDF accessibility may not be a problem in vivo. Studies using live animals will resolve these issues.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

References

1. Li F, Song N, Tombran-Tink J, Niyibizi C (2015) Pigment epithelium derived factor suppresses expression of Sost/sclerostin by osteocytes: implication for its role in bone matrix mineralization. J Cell Physiol 24:1651–1661
2. Dawson DW, Volpert OV, Gillis P, Crawford SE, Xu H, Benedict W, Bouck NP (1999) Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. Science 285:245–248
3. Tombran-Tink J, Chader GG, Johnson LV (1991) PEDF: a pigment epithelium-derived factor with potent neuronal differentiative activity. Exp Eye Res 53:411–414
4. Steele FR, Chader GJ, Johnson LV, Tombran-Tink J (1993) Pigment epithelium-derived factor: neurotrophic activity and identification as a member of the serine protease inhibitor gene family. Proc Natl Acad Sci U S A. 90:1526–1530
5. Ho TC, Chen SL, Shih SC, Wu JY, Han WH, Cheng HC, Yang SL, Tsao YP (2010) Pigment epithelium-derived factor is an intrinsic antifibrosis factor targeting hepatic stellate cells. Am J Pathol 177:1798–1811
6. Cai J, Jiang WG, Grant MB, Boulton M (2006) Pigment epithelium-derived factor inhibits angiogenesis via regulated intracellular proteolysis of vascular endothelial growth factor receptor 1. J Biol Chem 281:3604–3613
7. Tombran-Tink J (2010) PEDF in angiogenic eye diseases. Curr Mol Med 10:267–278
8. Becker J, Semler O, Gilissen C, Li Y, Bolz HJ, Giunta C, Bergmann C, Rohrbach M, Koerber F, Zimmermann K, de Vries P, Wirth B, Schoenau E, Wollnik B, Veltman JA, Hoischen A, Netzer C (2011) Exome sequencing identifies truncating mutations in human SERPINF1 in autosomal-recessive osteogenesis imperfecta. Am J Hum Genet 88:362–371
9. Homan EP, Rauch F, Grafe I, Lietman C, Doll JA, Dawson B, Bertin T, Napierala D, Morello R, Gibbs R, White L, Miki R,

Cohn DH, Crawford S, Travers R, Glorieux FH, Lee B (2011) Mutations in SERPINF1 cause osteogenesis imperfecta type VI. J Bone Miner Res 26:2798–2803
10. Venturi G, Gandini A, Monti E, Dalle Carbonare L, Corradi M, Vincenzi M, Valenti MT, Valli M, Pelilli E, Boner A, Mottes M, Antoniazzi F (2012) Lack of expression of SERPINF1, the gene coding for pigment epithelium-derived factor, causes progressively deforming osteogenesis imperfecta with normal type I collagen. J Bone Miner Res 27:723–728
11. Li F, Song N, Tombran-Tink J, Niyibizi C (2013) Pigment epithelium-derived factor enhances differentiation and mineral deposition of human mesenchymal stem cells. Stem Cells 31:2714–2723
12. Gattu AK, Swenson ES, Iwakiri Y, Samuel VT, Troiano N, Berry R, Church CD, Rodeheffer MS, Carpenter TO, Chung C (2013) Determination of mesenchymal stem cell fate by pigment epithelium-derived factor (PEDF) results in increased adiposity and reduced bone mineral content. FASEB J 27:4384–4394
13. Belinsky GS, Sreekumar B, Andrejecsk JW, Saltzman WM, Gong J, Herzog RI, Lin S, Horsley V, Carpenter TO, Chung C (2016) Pigment epithelium-derived factor restoration increases bone mass and improves bone plasticity in a model of osteogenesis imperfecta type VI via Wnt3a blockade. FASEB J 30:2837–2848
14. Sekiya H, Mikuni-Takagaki Y, Kondoh T, Seto K (1999) Synergistic effect of PTH on the mechanical responses of human alveolar osteocytes. Biochem Biophys Res Commun 264:719–723
15. Gu G, Nars M, Hentunen TA, Metsikko K, Vaananen HK (2006) Isolated primary osteocytes express functional gap junctions in vitro. Cell Tissue Res 323:263–271
16. Stern AR, Stern MM, Van Dyke ME, Jahn K, Prideaux M, Bonewald LF (2012) Isolation and culture of primary osteocytes from the long bones of skeletally mature and aged mice. Biotechniques 52:361–373
17. Abubakar AA, Noordin MM, Azmi TI, Kaka U, Loqman MY (2016) The use of rats and mice as animal models in ex vivo bone growth and development studies. Bone Joint Res 5:610–618
18. Okubo N, Minami Y, Fujiwara H, Umemura Y, Tsuchiya Y, Shirai T, Oda R, Inokawa H, Kubo T, Yagita K (2013) Prolonged bioluminescence monitoring in mouse ex vivo bone culture revealed persistent circadian rhythms in articular cartilages and growth plates. PLoS One 8:e78306
19. Chan ME, Lu XL, Huo B, Baik AD, Chiang V, Guldberg RE, Lu HH, Guo XE (2009) A trabecular bone explant model of osteocyte-osteoblast co-culture for bone mechanobiology. Cell Mol Bioeng 2:405–415
20. Marino S, Staines KA, Brown G, Howard-Jones RA, Adamczyk M (2016) Models of ex vivo explant cultures: applications in bone research. BoneKEy Rep 5:818
21. Li F, Cain J, Tombran-Tink J, Niyibizi C (2018) Pigment epithelium derived factor regulates human Sost/Sclerostin and other osteocyte gene expression via the receptor and induction of Erk/Gsk-3beta/beta-catenin signaling. Biochem Biophy Acta Mol Basis Dis 1864:3449–3458
22. Atkins GJ, Rowe PS, Lim HP, Welldon KJ, Ormsby R, Wijenayaka AR, Zelenchuk L, Evdokiou A, Findlay DM (2011) Sclerostin is a locally acting regulator of late-osteoblast/preosteocyte differentiation and regulates mineralization through a MEPE-ASARM-dependent mechanism. J Bone Miner Res 26:1425–1436
23. Winkler DG, Sutherland MK, Geoghegan JC, Yu C, Hayes T, Skonier JE, Shpektor D, Jonas M, Kovacevich BR, Staehling-Hampton K, Appleby M, Brunkow ME, Latham JA (2003) Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. EMBO J 22:6267–6276
24. Paszty C, Turner CH, Robinson MK (2010) Sclerostin: a gem from the genome leads to bone-building antibodies. J Bone Miner Res 25:1897–1904
25. Tian X, Jee WS, Li X, Paszty C, Ke HZ (2011) Sclerostin antibody increases bone mass by stimulating bone formation and inhibiting bone resorption in a hindlimb-immobilization rat model. Bone 48:197–201
26. Lin C, Jiang X, Dai Z, Guo X, Weng T, Wang J, Li Y, Feng G, Gao X, He L (2009) Sclerostin mediates bone response to mechanical unloading through antagonizing Wnt/beta-catenin signaling. J Bone Miner Res 24:1651–1661
27. Addison WN, Nakano Y, Loisel T, Crine P, McKee MD (2008) MEPE-ASARM peptides control extracellular matrix mineralization by binding to hydroxyapatite: an inhibition regulated by PHEX cleavage of ASARM. J Bone Miner Res 23:1638–1649
28. Martin A, David V, Laurence JS, Schwarz PM, Lafer EM, Hedge AM, Rowe PS (2008) Degradation of MEPE, DMP1, and release of SIBLING ASARM-peptides (minhibins): ASARM-peptide(s) are directly responsible for defective mineralization in HYP. Endocrinology 149:1757–1772
29. David V, Martin A, Hedge AM, Rowe PS (2009) Matrix extracellular phosphoglycoprotein (MEPE) is a new bone renal hormone and vascularization modulator. Endocrinology 150:4012–4023
30. Lu P, Zhang YQ, Zhang H, Li YF, Wang XY, Xu H, Liu ZW, Li L, Dong HY, Zhang ZM (2016) Pigment epithelium-derived factor (PEDF) improves ischemic cardiac functional reserve through decreasing hypoxic cardiomyocyte contractility through PEDF receptor (PEDF-R). J Am Heart Assoc 5:e003179
31. Gowen LC, Petersen DN, Mansolf AL, Qi H, Stock JL, Tkalcevic GT, Simmons HA, Crawford DT, Chidsey-Frink KL, Ke HZ, McNeish JD, Brown TA (2003) Targeted disruption of the osteoblast/osteocyte factor 45 gene (OF45) results in increased bone formation and bone mass. J Biol Chem 278:1998–2007
32. Staines KA, Pollard AS, McGonnell IM, Farquharson C, Pitsillides AA (2013) Cartilage to bone transitions in health and disease. J Endocrinol 219:R1–R12