Supplementary Methods

Generation of conditional *Pdgfb* knockout and transgenic mice

The *Pdgfb*$^{f/f}$ mouse strain was purchased from the Jackson Laboratory. The *Trap-Cre* mouse strain(1) was kindly provided by Jolene J. Windle (Virginia Commonwealth University, Richmond, VA). Hemizygous *Trap-Cre* mice were crossed with *Pdgfb*$^{f/f}$ mice. The offspring were intercrossed to generate the following offspring: WT, *Trap-Cre* (mice expressing Cre recombinase driven by *Trap* promoter), *Pdgfb*$^{f/f}$ (mice homozygous for *Pdgfb* flox allele, which are referred to as "WT" in the text because the mice were used in the experiments as WT littermate controls), and *TRAP-Cre*; *Pdgfb*$^{f/f}$ (mice with *Pdgfb* conditional deletion in Trap lineage cells, which are referred to as "Pdgfb^{cKO}" in the text). We determined the genotype of the mice by polymerase chain reaction analyses of genomic DNA isolated from mouse tails using the following primers: *Trap*-directed *Cre* forward, 5′-ATATCTCACGTACTGACGGTGGG-3′ and reverse, 5′-CTGTTTCACTATCCAGGTTACGG-3′; *loxP Pdgfb* allele forward, 5′-GGGTGGGACTTTGGTGTAGAGAAG-3′ and reverse, 5′-GGAACGGATTTTGGAGGTAGTGTC-3′. *TRAP-cre* mice were crossed to tdTomato reporter mouse (Jax stock# 007914) to generate *TRAP/tdTom* mice that express the tdTomato under *TRAP-cre*. Images of the fluorescent tdTomato for the detection of cre recombination were taken using Zeiss LSM 780 confocal microscope.

Conditional transgenic mice, Pdgfb^{cTG}, were generated as described previously(2) . Briefly, mouse TRACP5 promoter was ligated with 2.8-kb full-length human Pdgfb complementary

DNA. Transgenic mice were produced by pronuclear injection of C57BL/6 fertilized eggs at the Transgenic Mouse Core facility. All animals were housed in the animal facility at the Johns Hopkins University. The experimental protocol was reviewed and approved by our Institutional Animal Care and Use Committee.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA for qRT-PCR was extracted from the sorting cells using RNeasy Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. Complementary DNA was prepared with random primers using the SuperScript First-Strand Synthesis System (Invitrogen, Waltham, MA) and analyzed with Fast SYBR Green Master Mix (Thermo Fisher Scientific, Waltham, MA) in the thermal cycler with two sets of primers specific for each targeted gene. Target-gene expression was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA, and relative gene expression was assessed using the $2^{-\Delta\Delta CT}$ method. The primers used for qRT-PCR were as follows: Pdgfb 5'-CATCCGCTCCTTTGATGATCTT-3' and 5'- GTGCTCGGGTCATGTTCAAGT-3'; ALP (5'-CCAACTCTTTTGTGCCAGAGA-3' and 5'-GGCTACATTGGTGTTGAGCTTTT-3'), RUNX2 (5'-AGAGTCAGATTACAGATCCCAGG-3' and 5'-TGGCTCTTCTTACTGAGAGAGG-3'), GAPDH (5′-CTGCTTCACCACCTTCTTGA -3′) and (5′-AAGGTCATCCCAGAGCTGAA -3′).

Bone marrow supernatant collection and enzyme-linked immunosorbent assay (ELISA) analysis

Mouse bone marrow supernatant was collected using a method described previously(3). Briefly, tibial bones were harvested from the euthanized mice, and the two ends of the bone were cut to expose bone marrow. The bone was then placed into an Eppendorf tube and subjected to centrifugation for 15 min at 3,000 rpm and 4°C to obtain bone marrow supernatants. PDGF-BB ELISA in serum or bone marrow supernatant was performed using the Mouse/Rat PDGF-BB Quantikine ELISA Kit (MBB00, R&D Systems, Inc., Minneapolis, MN) according to the manufacturer's instructions.

Preparation of CM from preosteoclasts and in vitro VSMCs assays

Different CM from MOs/MACs, mononuclear preosteoclasts, and multinucleated mature osteoclasts were prepared as previously described(3). In some experiments, neutralizing antibody for PDGF-BB (Abcam, 1 μg ml^{-1}, Polyclonal) was added to the CM. The isolation and culture of the rat primary VSMCs were performed as described previously(4). Cell proliferation was conducted using MTT assays. Briefly, VSMCs were plated onto 96-well plates and treated with different CM. After 48 h, 2,5-diphenyltetrazolium bromide (MTT) solution was added to the media, and the cells were incubated for 3 h. After 3h, stop solution were add in to each well and incubated for overnight. The MTT-containing medium was discarded, and the cells were gently washed with PBS. Formazan that formed within the cells was then dissolved in acetic-isopropanol, and the plate was read at 570 nm with correction at 750 nm. The optical density of the treatments was normalized to the group with control medium. Cell migration was performed using transwell assays. Briefly, VSMCs were placed in the upper chambers of the transwell, and the CM was placed in the lower chambers. At the

end of incubation, we fixed the cells with 10% formaldehyde for 30 min and then removed the cells on the upper surface of each filter with cotton swabs. We stained the cells that had migrated through the pores to the lower surface with crystal violet (Sigma-Aldrich) and quantified them by counting five random fields per well using a microscope (Olympus, Tokyo, Japan) at ×200 magnification.

Reference

1. Dossa T, et al. Osteoclast-specific inactivation of the integrin-linked kinase (ILK) inhibits bone resorption. *J Cell Biochem.* 2010;110(4):960-7.
2. Su W, et al. Angiogenesis stimulated by elevated PDGF-BB in subchondral bone contributes to osteoarthritis development. *JCI Insight.* 2020;5(8).
3. Xie H, et al. PDGF-BB secreted by preosteoclasts induces angiogenesis during coupling with osteogenesis. *Nat Med.* 2014;20(11):1270-8.
4. Joshi CN, et al. Control of vascular smooth muscle cell growth by connexin 43. *Front Physiol.* 2012;3:220.