Original Paper

Endothelial Progenitor Cells Enhance the Migration and Osteoclastic Differentiation of Bone Marrow-Derived Macrophages *in vitro* and in a Mouse Femur Fracture Model through Talin-1

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Abstract
Background/Aims: Bone resorption mediated by osteoclasts plays an important role in bone healing. Endothelial progenitor cells (EPCs) promote bone repair by stimulating neovascularization and osteogenesis. However, the role of EPCs in osteoclast formation and function is not well defined. The aim of this study was to elucidate mechanisms of EPCs in osteoclast formation and function. ***Methods:*** In this study, we examined the effects of EPCs on the proliferation, migration and osteoclastic differentiation of primary mouse bone marrow-derived macrophages (BMMs) in a co-culture system *in vitro*. We also evaluated the effects of EPC co-transplantation on the homing and osteoclastic differentiation of transplanted BMMs in a mouse bone fracture model *in vivo*. The technology of immunofluorescence, immunohistochemical, western blot, Rt-PCR, cell co-culture and Transwell were used in this study. ***Results:*** EPCs secreted TGF-β1 in the EPC-BMM co-culture medium and increased Talin-1 expression in the co-cultured BMMs. Treatment with a TGF-β1 neutralizing antibody or Talin-1 silencing in BMMs completely inhibited BMM osteoclastic differentiation in the co-culture system. These results indicated that the osteoclastogenic effects of EPCs were mediated by TGF-β1-mediated Talin-1 expression in BMMs. In the femur fracture model, BMMs co-transplanted with EPCs exhibited enhanced engraftment into the fracture site and osteoclastic differentiation compared with those transplanted alone. Mice treated with EPC-BMM co-transplantation exhibited increased neovascularization at the fracture site and accelerated fracture healing compared with those treated with BMMs alone. ***Conclusion:*** Taken

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together, the results suggest that EPCs can promote bone repair by enhancing recruitment and differentiation of osteoclast precursors.

Introduction

Bone is one of the few tissues/organs that can undergo complete regeneration after injury [1]. Bone healing occurs through the coordinated activities of osteoclasts and osteoblasts to achieve site-directed removal of damaged bone by osteoclasts and subsequent bone formation by osteoblasts to replace the bone loss [2]. Osteoclasts are highly specialized, multinucleated cells that are derived from hematopoietic precursors of the monocyte-macrophage lineage residing in the bone marrow [3]. Osteoclast formation (osteoclastogenesis) depends on two essential cytokines: RANKL (receptor activator of nuclear factor (NF)-κB ligand) and M-CSF (macrophage colony-stimulating factor). RANKL, a tumor necrosis factor (TNF) family cytokine, directly controls osteoclast differentiation through activation of TNF receptor-associated factor (TRAF) 6, NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and c-Fos [4]. M-CSF supports the survival and proliferation of the osteoclast precursor cells [5, 6] through activation of Mitf and Bcl-2 [7, 8]. The full differentiation and activation of osteoclasts require several additional signals that argument RANKL or M-CSF signaling [9]. For instance, TGF-β has been reported to promote RANKL-induced osteoclastogenesis through SMAD2/SMAD3 [10], and β3 integrins have been shown to regulate the differentiation and function of osteoclasts in concert with M-CSF [11, 12].

Endothelial progenitor cells (EPCs) give rise to functional endothelial cells to sustain vasculogenesis. They home to sites of vascular injury to engage in angiogenesis during the process of tissue remodeling and regeneration [13]. EPCs can promote neovascularization of tissue-engineered bones, and further improve osteogenesis and bone reconstruction [14-16]. In addition, they can stimulate osteoblastic differentiation *in vitro* [17] and accelerate fracture healing in a segmental bone defect model *in vivo* [18]. Emerging evidence suggests that EPCs may also play a role in osteoclast formation and function. Specifically, EPCs have been reported to support the survival, migration, and differentiation of RAW 264.7 cells, a model of osteoclastic monocyte precursors, through multiple signaling pathways [19, 20]. However, whether EPCs can influence osteoclastogenesis by bone marrow-derived macrophages (BMMs) is not clear.

β3 integrin signaling is a well-known mechanism regulating the formation and activity of osteoclasts [12, 21]. Integrins use numerous cytosolic integrin-associated proteins (IAPs) to mediate their functions. Talin is the most important IAP that links β integrins directly to actin, increasing the affinity of integrin for ligands and activating downstream events [22]. Studies have shown that Talin-1 plays a critical in the motility and bone resorption of osteoblasts [23, 24]. In this study, we investigated the role of EPCs in the survival, migration and osteoclastic differentiation of BMMs *in vitro*. We also evaluated the effects of EPC co-transplantation on the homing and osteoclastic differentiation of transplanted BMMs in a mouse bone fracture model *in vivo*. The underlying molecular mechanisms involving TGF-β and Talin-1 were examined.

Materials and Methods

Animals

Six-week-old C57BL/10 mice were purchased from the Model Animal Research Center (MARC) of Nanjing University. All animal experiments were performed in accordance with the Institutional Guidelines for the Care and Use of Laboratory Animals of the Southwest Hospital, affiliated with the Third Military Medical University. All study protocols were reviewed and approved by the Animal Ethics Committee of the Southwest Hospital.

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Isolation and characterization of EPCs

Mononuclear cells were isolated from mouse umbilical cord blood. Cells expressing the early EPC surface marker CD133 were selected using anti-CD133-coupled magnetic microbeads (Miltenyi Biotech, Bergisch Gladbach, Germany) and expanded in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Immunophenotypic analysis was performed by staining 5×10^5 cells with monoclonal antibodies against CD31, CD34, CD133, vWF, and UFA-1, respectively (Sigma, St. Louis, MO, USA). Isotype-identical antibodies served as controls (PharMingen, San Diego, CA, USA). All cells were counterstained with the carbocyanine fluorescent dye Dil (Molecular Probes, Eugene, OR, USA). The angiogenic capacity of the early EPCs was determined by the Matrigel tube formation assay as we previously reported [19]. In brief, Matrigel (Sigma) was diluted in 500 µl EGM-2 media (1:1 v/v) in 96-well plates and incubated at 37°C for 1 h to allow polymerization. EPCs (2×10^4 cells/well) were seeded onto the Matrigel and incubated at 37°C for 24 h. Digital micrographs were taken for morphological analysis.

BMM isolation and differentiation

Mouse primary BMMs were prepared as previously described [24] with minor modifications. In brief, marrow was extracted from femora and tibiae of six-week-old C57BL/10 mice with α-minimal essential medium (α-MEM) and placed in Petri dishes in a culture medium composed of α-MEM supplemented with 10% inactivated fetal bovine serum (FBS), 100 IU/ml penicillin and 100 µg/ml streptomycin and CMG medium (conditioned medium containing 100 ng/ml mouse M-CSF) in a 1:10 ratio. Cells were incubated at 37°C with 5% CO_2 for 3 days, washed with phosphate-buffered saline (PBS) and harvested with trypsin/EDTA (Invitrogen, Carlsbad, CA, USA).

To test the effects of EPCs on the proliferation and osteoclastic differentiation of BMMs, a co-culture system was established by inserting transwell chambers with 3 µm pores (Millipore, Bedford, MA, USA) pre-seeded with EPCs (1×10^3 cells/chamber) into 12-well culture plates pre-seeded with BMMs (1×10^3 cells/well). DMEM (1 ml/well) was subsequently added to cover both the EPCs and BMMs to allow crosstalk between the two cell types. Half of the medium was removed and replaced with fresh medium every two days. Cell proliferation was determined using the Cell Counting Kit-8 (CCK-8) assay. Osteoclastic differentiation was assessed by immunofluorescence staining for the osteoclast marker TRAP (tartrate acid resistant phosphatase) using anti-TRAP antibody (Sigma-Aldrich, St. Louis, MO, USA). BMMs cultured in medium containing GST-RANKL (100 ng/ml) and M-CSF (30 ng/ml) served as positive control for osteoclastic differentiation. TGF-β1 levels in culture supernatants were determined by ELISA (Sigma-Aldrich, St. Louis, MO, USA). To test the effects of TGF-β1 inhibition, cells were cultured in medium containing a neutralizing antibody toward TGF-β1 (Sigma).

Transwell migration assay

The migration capacity of BMMs was measured using transwell inserts with a pore size of 8 µm (Costar, Corning, NY). In brief, BMMs were loaded into the upper chamber and EPCs into the lower chamber. After incubation at 37°C with 5% CO_2 for 5 days, the BMMs were rinsed with PBS, fixed in 10% formalin for 10 min, and stained with DAPI for 15 min. The cells that had migrated to the lower surface of the membrane were counted in three randomly selected fields under an inverted light microscope (Leica, Germany) as previously described [25].

RNA interference

BMMs were collected and resuspended in Electroporation Isoosmolar Buffer (Eppendorf). The cells were transfected using electroporation with a Stealth RNAi™ small interfering RNA (siRNA) targeting Talin-1 (sense sequence: CAGCUCAUUGCUGGCUACAUAGAUA, Invitrogen) or a Stealth RNAi™ siRNA negative control (Med GC) on an ECM830 Electro-Square Porator (Harvard Apparatus). The electroporation was carried out using a single square wave pulse of 2, 500 V/cm field strength with 300 µs pulse length. After that, the cells were allowed to recover for 46 h in DMEM.

Western blot analysis

Cells were lysed and total proteins were quantified. Samples (30-50 µg each) were separated by 10% SDS-PAGE (stacking gel 50 V, separating gel 100 V) and transferred to nitrocellulose membranes (100 V for

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75 min). After blocking in defatted milk for 2 h, the membranes were incubated with primary antibodies followed by horse radish peroxidase (HRP)-conjugated secondary antibodies. The protein bands were visualized using enhanced chemiluminescence. Densitometry analysis of protein levels was performed using Gel-pro Image Analysis Software (Media cybernetics, Rockville, MD, USA).

Preparation of green fluorescent protein (GFP)-labeled EPCs

EPCs were seeded in 24-well plates (5×10^4 cells/well) and cultured until they reached 70% confluence. The cells were subsequently incubated with lentiviral vectors expressing GFP (Invitrogen) at a multiplicity of infection (MOI) of 20. The medium was replaced after 24 h, and the fluorescence intensity was recorded after 96 h. Uninfected EPCs served as negative control.

Femur fracture model

All surgical procedures were performed under normal aseptic conditions. Mice were anesthetized with intraperitoneal ketamine hydrochloride (60 mg/kg) and xylazine hydrochloride (10mg/kg). Femur fractures (unilateral) were produced by 3-point bending using the method described by Manigrasso [26]. Unfractured femurs served as negative control. Immediately after the fracture, mice received intravenous injection of BMMs (2×10^5 cells) suspended in Endothelial Basal Medium-2 (EBM-2, Lonza), either alone or in combination with GFP-labeled EPCs (2×10^5 cells). To detect BMM homing to the fracture site, the cells were labeled with Dil (Molecular Probes) at 2.5 mg/ml for 5 min at 37°C followed by another 15 min at 4°C just before transplantation. To assess angiogenesis by EPCs, tissue samples were collected from the fracture site on day 7 or day 28 after cell transplantation, embedded in OCT compound (Sakura Finetek Japan, Tokyo, Japan), snap-frozen in liquid nitrogen and cut into 5-μm-thick sections. The sections were stained with anti-CD31 antibody (Vector Laboratories, Burlingame, CA, USA), counterstained with DAPI, and examined under a fluorescence microscope. Dil-positive capillaries were counted in 10 randomly selected high-power fields. Talin-1 expression in BMMs was detected by immunofluorescence staining with anti-Talin-1 antibody (Vector Laboratories, Burlingame, CA, USA).

Microcomputed tomography (μCT)

The trabecular volumes at the distal femoral metaphysis and proximal tibia were determined using a Scanco CT40 scanner (Scanco Medical AG, Bassersdorf, Switzerland; threshold = 250). Forty to 100 slices were analyzed, starting with the first slice in which condyles and primary spongiosa were no longer visible.

Quantitative real-time PCR analysis

RNA was isolated from BMMs or bone tissues using TRIZOL (Invitrogen). cDNA was synthesized from 1 μg of total RNA in 21-μl reaction volumes using oligo dT18 primers and SuperScript reverse transcriptase. PCR amplification was carried out with Taq DNA polymerase (TaKaRa) using 1 μl of the first-strand cDNAs as templates. The amplification reactions were run with 30 thermocycles of 30 s at 94°C, 30 s at 55°C and 30 s at 72°C. The primer sequences were: Car2, 5'-CATTACTGTCAGCAGCGAGCA-3' (sense) and 5'-GACGCCAGTTGTCCACCATC-3' (anti-sense); CTSK, 5'-CAGCAGAACGGAGGCATTGA-3' (sense) and 5'-CCTTTGCCGTGGCGTTATAC-3' (anti-sense); MMP9, 5'-GCCCTGGAACTCACACGACA-3' (sense) and 5'-TTGGAAACTCACACGCCAGAA-3' (anti-sense); TRAP, 5'-CAGTTGGCAGCAGCCAAGGAGGAC-3' (sense) and 5'-GTCCCTCAGGAGTCTAGGTATCAC-3' (anti-sense); GAPDH, 5'-CACAGTCAAGGCCGAGAATG-3' (sense) and 5'-TCGTGGTTCACACCCATCAC-3' (anti-sense).

Statistical analysis

All data are reported as the mean ± SD. The Student's two-tailed unpaired *t*-test was applied to interpret differences between two groups. A p value less than 0.05 was regarded as statistically significant.

Results

EPCs enhance the proliferation, migration and osteoclastic differentiation of co-cultured BMMs

The CD133$^+$ cells isolated from mouse umbilical cord blood exhibited a spindle-like morphology (Fig. 1A) and expressed a spectrum of EPC-specific surface markers including CD31, CD34, CD133, Flk-1, VEGFR2, vWF and UFA-1 (Fig. 1B–H). These cells also showed angiogenic potential as they formed tube-like structures on Matrigel (Fig. 1I). Compared with BMMs cultured alone, BMMs co-cultured with EPCs exhibited significantly increased cell proliferation and migration capacity (Fig. 2A–D). We subsequently assessed osteoclastic differentiation of BMMs by immunofluorescence staining for the osteoclast marker TRAP [27]. We found that co-culture with EPCs greatly enhanced the differentiation of BMMs into TRAP-positive multinucleated osteoclast-like cells, although the osteoclastogenic activity of EPCs was less potent compared with RANKL plus M-SCF (Fig. 3A, B).

EPCs enhance osteoclastic differentiation of co-cultured BMMs through TGF-β1-mediated Talin-1 signaling

ELISA revealed increased TGF-β1 level in the EPC-BMM co-culture supernatant compared with BMM culture alone (Fig. 4A). Blocking TGF-β1 signaling with a TGF-β1 neutralizing antibody inhibited the differentiation of the co-cultured BMMs into TRAP-positive multinucleated osteoclast-like cells (Fig. 4B, C). Western blot analysis detected

Fig. 1. Culture and characterization of EPCs. (A–H) Representative microscopic images of EPCs (A) and immunofluorescence staining for the specific cell surface markers CD31 (B), CD34 (C), CD133 (D), Flk-1 (E), VEGFR2 (F), vWF (G) and UFA-1 (H) after 7 days of growth in culture, magnification 400×. (I) EPCs formed tube-like structures after 24 h of growth on Matrigel.
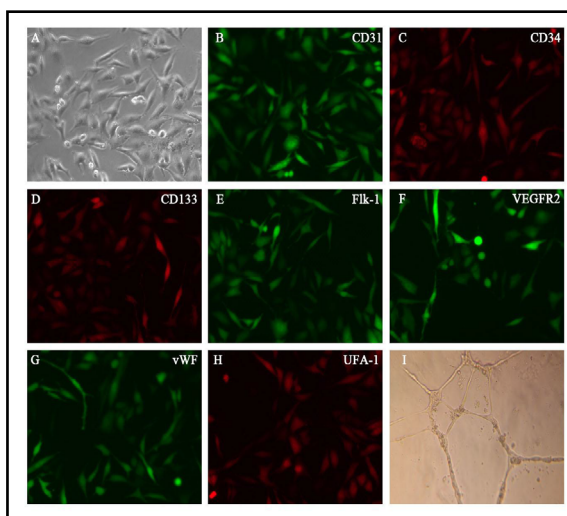

Fig. 2. EPCs enhance the proliferation and migration of co-cultured BMMs. (A–B) Representative microscopic images of BMMs after 7 days of growth (A, magnification 400×) and cell proliferation determined by the CCK-8 assay after 1, 3, 5 or 7 days of growth (B), with the cells either cultured alone (control) or co-cultured with EPCs (co-culture). n = 5; *p<0.05, **p<0.01, ***p<0.001 vs. control. (C–D) Representative images of BMMs (stained with crystal violet) on the lower surface of the transwell membrane (C) and quantitative analysis of cell migration by cell counting (D) after 5 days in culture, with the cells either cultured alone (control) or co-cultured with EPCs (co-culture). n = 3, ***p<0.001 vs. control.
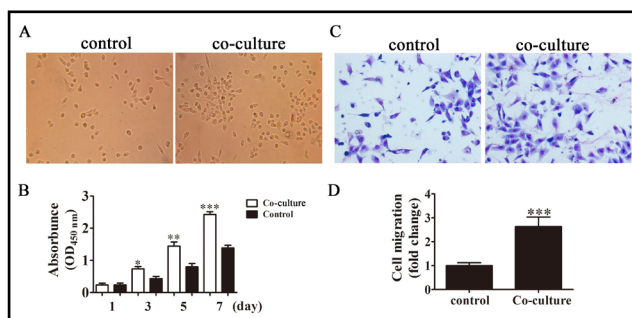
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Fig. 3. EPCs promote osteoclastic differentiation of co-cultured BMMs. (A–B) Representative immunofluorescence images of TRAP (A, magnification 200×) and the numbers of TRAP-positive multinucleated (≥ 3 nuclei) cells (B) of BMMs after 7 days of

culture, with the cells either cultured alone (control), co-cultured with EPCs (co-culture), or cultured with GST-RANKL (100 ng/ml) and M-CSF (30 ng/ml) (RANKL+M-CSF). n = 3, ***p<0.001 vs. control, ###p<0.001 vs. co-culture. The arrows indicate TRAP-positive multinucleated osteoclast-like cells.
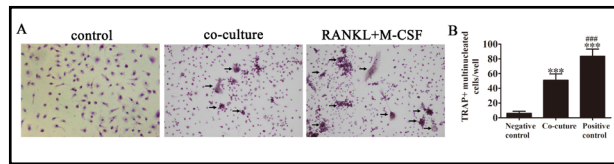
Fig. 4. EPC-derived TGF-β1 promotes BMM osteoclastic differentiation through Talin-1. (A) TGF-β1 levels in BMM culture supernatants by ELISA. BMMs were either cultured alone (control) or co-cultured with EPCs (co-culture) for 7 days. (B–D) The numbers of TRAP-positive multinucleated (≥ 3 nuclei) cells (B), representative immunohistochemical images of TRAP (C) and Talin-1 expression (Western blot analysis) (D) of BMMs after 7 days of culture. The cells were either cultured alone (control), co-cultured with EPCs (co-culture) or co-cultured with EPCs in the presence of a TGF-β1 neutralizing antibody (TGF-β1 inhibitor). (E,F) The numbers of TRAP-positive multinucleated (≥ 3 nuclei) cells (E) and representative immunohistochemical images of TRAP (F) of BMMs after 7 days of culture. The cells were either cultured alone (control), co-cultured with EPCs (co-culture) or transfected with a Talin-1-targeting siRNA prior to co-culture with EPCs (siTalin-1). n = 3, ***p<0.001 vs. control, ###p<0.001 vs. co-culture.
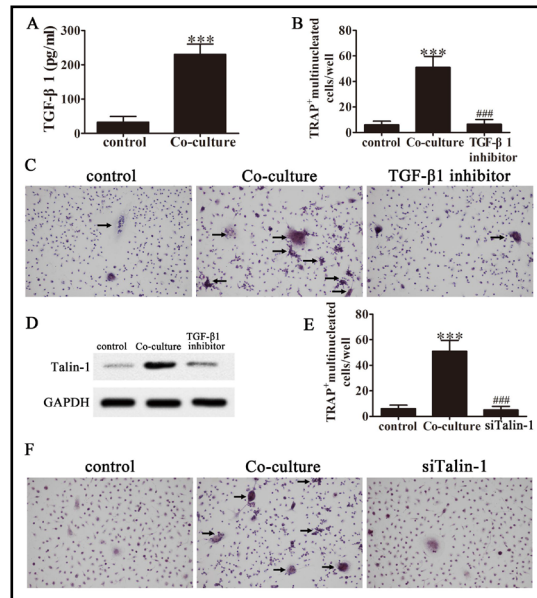
increased Talin-1 expression in the co-cultured BMMs compared with BMMs cultured alone, and incubation with the TGF-β1 neutralizing antibody blocked Talin-1 expression in the co-cultured BMMs (Fig. 4D). Similar to treatment with the TGF-β1 neutralizing antibody, Talin-1 knockdown by siRNA transfection in BMMs prevented the osteoclastic differentiation of the co-cultured BMMs (Fig. 4E, F). The result show that compared with control or negative control (NC). The expression of Talin-1 was signifigantly decreased in both protein and mRNA level (Fig. 5). In addition, compared with BMMs cultured alone, BMMs co-cultured with EPCs exhibited increased mRNA and protein expression of the osteoclast genes MMP9, CTSK, TRAP, and Car2, and treatment with the TGF-β1 neutralizing antibody or Talin-1 knockdown prevented the expression of these osteoclast genes (Fig. 6A–E). Collectively, these data indicated that EPCs promote osteoclastic differentiation of the co-cultured BMMs through TGF-β1 secretion and consequent upregulation of BMM Talin-1 expression.

EPC co-transplantation promotes the homing and osteoclastic differentiation of BMMs, and further accelerates bone healing

Four weeks after cell transplantation, femur fractures treated with Dil-labeled BMMs along with GFP-labeled EPCs exhibited better bone healing than those treated with Dil-labeled BMMs alone (Fig. 7A). Transplantation of BMMs alone led to partial fracture repair, while BMM-EPC co-transplantation resulted in formation of continuous new bone that covered the entire fracture area. Seven days after transplantation, mice treated with BMM-EPC co-transplantation showed a greater number of Dil-labeled BMMs with higher Talin-1 expression around the fracture site than those treated with BMM transplantation alone (Fig.

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Fig. 5. The expression of Talin-1 were detected after transfected with siRNA aganist Talin-1 vector. (A and B) Western blot (A) and qRt-PCR (B) detection the expression of Talin-1 after transfected with siRNA against Talin-1 for 48 h.
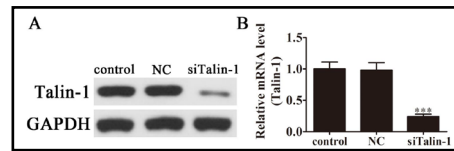

Fig. 6. EPCs promote osteoclast gene expression in co-cultured BMMs through TGF-β1-mediated Talin-1 signaling. (A–E) The mRNA levels of the osteoclast genes MMP9 (A), CTSK (B), TRAP (C) and Car2 (D) by quantitative RT-PCR and the protein levels of these genes by western blot analysis (E) in BMMs after 7 days of culture. The cells were either cultured alone (control), co-cultured with EPCs (co-culture), co-cultured with EPCs in the presence of a TGF-β1 neutralizing antibody (TGF-β1 inhibitor) or transfected with a Talin-1-targeting siRNA prior to co-culture with EPCs (siTalin-1). GAPDH was used as controls. n = 3; **p<0.01, ***p<0.001 vs. control.
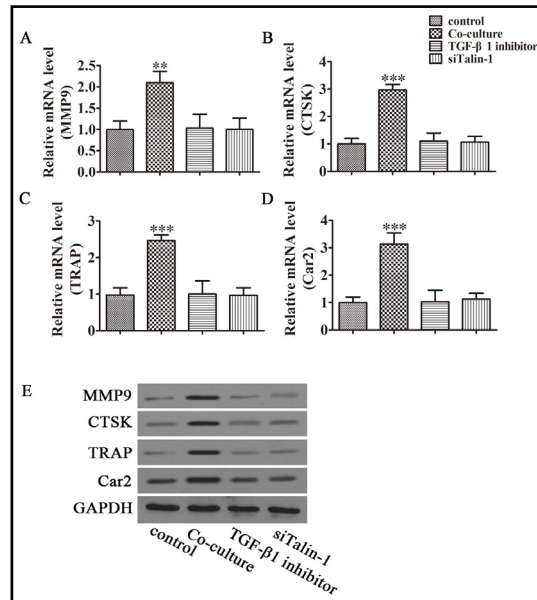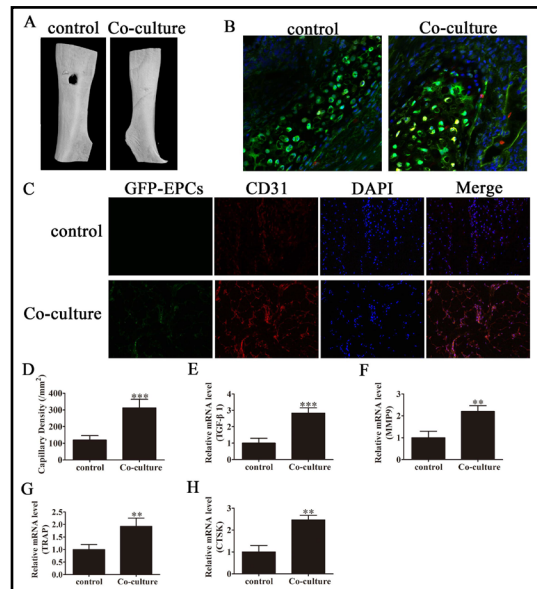

Fig. 7. EPC co-transplantation promotes the homing and osteoclastic differentiation of transplanted BMMs, and further accelerates bone healing. Mice received intravenous transplantation of Dil-labeled BMMs, either alone (control) or in combination with GFP-labeled EPCs (co-culture), immediately after femur fracture. (A) Representative μCT images of the fractured femur at week 4. (B) Immunofluorescence staining for Talin-1 expression in Dil-labeled BMMs around the fracture site at day 7. (C) Representative fluorescence images of GFP and immunofluorescence images of CD31 of tissues at the fracture site at week 4. Tissues were counterstained with DAPI. (D) Density of Dil-positive capillaries at the fracture site at week 4. (E–H) The relative mRNA levels of TGF-β1 (E), MMP9 (F), TRAP (G) and CTSK (H) in tissues at the fracture site at day 7 by qRT-PCR. n = 3; **p<0.01, ***p<0.001 vs. control.

7B), indicating that EPC co-transplantation promoted the homing of the transplanted BMMs to the injury site and stimulated Talin-1 expression in engrafted cells. The BMM-EPC co-transplantation group also exhibited increased CD31 immunofluorescence at the fracture site 4 weeks after transplantation, especially in areas where GFP-labeled EPCs were detected (Fig. 7C). Interestingly, significant amounts of engrafted BMMs were detected around newly-formed capillaries at the fracture site in both treatment groups, with a higher density of such capillaries observed in the BMM-EPC co-transplantation group (Fig. 7D). These data suggested that the engrafted BMMs directly enhanced neovascularization at the fracture site

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by paracrine mechanisms. In keeping with our *in vitro* data, mice treated with BMM-EPC co-transplantation showed higher mRNA levels of TGF-β1 and the osteoclast genes MMP9, TRAP and CTSK at the fracture site than those treated with BMM transplantation alone (Fig. 7E–H).

Discussion

In animal models, EPCs have been shown to promote bone repair through a variety of mechanisms, by differentiating into endothelial cells and osteoblasts to enhance neovascularization and osteogenesis, as well as stimulating supporting cells by paracrine mechanisms through the secretion of growth factors and cytokines [28, 29]. They have also been reported to enhance osteogenic differentiation capacities of osteoblast precursors *in vitro* and *in vivo* [17, 30]. With regard to the role of EPCs in osteoclast formation and function, previous reports have shown that EPCs support the survival, migration, and differentiation of RAW 264.7 cells, a model of osteoclastic monocyte precursors [19, 20]. In this study, we found that EPCs derived from mouse umbilical cord blood promoted the proliferation, migration and osteoclastic differentiation of primary mouse BMMs in a EPC–BMM co-culture system. In keeping with these *in vitro* results, co-transplantation of EPCs enhanced the homing and osteoclastic differentiation of transplanted BMMs in a mouse femur fracture model, and further accelerated fracture healing.

TGF-β has been documented to regulate osteoclastogenesis by activating Smad2/3 [10]. In Pang's report [19], EPCs secreted VEGF-A, SDF-1 and TGF-β1 in the EPC–RAW 264.7 co-culture system and increased levels of phospho-Smad2/3, phospho-Akt, phospho-ERK1, and phospho-p38 MAPK in the co-cultured RAW 264.7 cells. These data supported the TGF-β1–Smad2/3 signaling pathway as a potential mediator of the osteoclastogenic effects of EPCs on the co-cultured RAW 264.7. In this study, EPCs secreted TGF-β1 in the EPC–BMM co-culture system, and the osteoclastogenic effects of EPCs on the co-cultured BMMs were completely blocked by treatment with a TGF-β1 neutralizing antibody. Taken together, these findings strongly suggest that EPCs stimulate differentiation of surrounding osteoclast precursors by paracrine mechanisms through the secretion of TGF-β1.

Talin-1 is a fundamental protein component of the osteoclast cytoskeleton, and as such plays a critical role in the adhesion, motility and bone resorption of osteoblasts [23, 24]. We found that EPCs increased Talin-1 expression in the co-cultured BMMs through a TGF-β1-depedent mechanism, and similar to TGF-β1 neutralizing antibody treatment, Talin-1 silencing in BMMs completed prevented osteoclastic differentiation of BMMs co-cultured with EPCs. Thus, Talin-1 acts downstream of TGF-β1 in mediating the osteoclastogenic effects of EPCs. The molecular mechanisms by which TGF-β1 increases Talin-1 expression in BMMs are currently unclear, and thus, require further investigation.

In the femur fracture model, BMMs co-transplanted with EPCs exhibited enhanced engraftment into the fracture site and osteoclastic differentiation compared with those transplanted alone. Co-transplantation of BMMs and EPCs also led to increased neovascularization at the injury site compared with BMM transplantation alone. We believe that the accelerated fracture healing achieved by the co-transplantation therapy was attributed to increased neovascularization as well as enhanced osteoclastic activity at the injury site.

Phase I/IIa clinical trials applying granulocyte colony stimulating factor-mobilized EPCs to nonunion bone fractures have generated accelerated fracture healing with minimal adverse effects [31]. Our findings shed new light on the mechanisms by which EPCs promote bone repair and support EPC-based therapy in orthopaedics.

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Disclosure Statement

The authors declare to have no competing interests.

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