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COMP-angiopoietin 1 increases proliferation, differentiation, and migration of stem-like cells through Tie-2-mediated activation of p38 MAPK and PI3K/Akt signal transduction pathways

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ABSTRACT

Recombinant COMP-Ang1, a chimera of angiopoietin-1 (Ang1) and a short coiled-coil domain of cartilage oligomeric matrix protein (COMP), is under consideration as a therapeutic agent capable of inducing the homing of cells with increased angiogenesis. However, the potentials of COMP-Ang1 to stimulate migration of mesenchymal stem cells (MSCs) and the associated mechanisms are not completely understood. We examined the potential of COMP-Ang1 on bone marrow (BM)-MSCs, human periodontal ligament stem cells (PDLSCs), and calvarial osteoblasts. COMP-Ang1 augmented Tie-2 induction at protein and mRNA levels and increased proliferation and expression of *runt-related transcription factor 2* (Runx2), *osterix*, and *CXCR4* in BMMSCs, but not in osteoblasts. The COMP-Ang1-mediated increases were inhibited by *Tie-2* knockdown and by treating inhibitors of phosphoinositide 3-kinase (PI3K), LY294002, or p38 mitogen-activated protein kinase (MAPK), SB203580. Phosphorylation of p38 MAPK and Akt was prevented by siRNA-mediated silencing of *Tie-2*. COMP-Ang1 also induced *in vitro* migration of BMMSCs and PDLSCs. The induced migration was suppressed by *Tie-2* knockdown and by CXCR4-specific peptide antagonist or LY294002, but not by SB203580. Furthermore, COMP-Ang1 stimulated the migration of PDLSCs into calvarial defect site of rats. Collectively, our results demonstrate that COMP-Ang1-stimulated proliferation, differentiation, and migration of progenitor cells may involve the Tie-2-mediated activation of p38 MAPK and PI3K/Akt pathways.

1. Introduction

Mesenchymal stem cells (MSCs) have generated much interest as a potential source of cells for cell-based therapeutic strategies. Bone marrow (BM)-derived MSCs (BMMSCs) are widely investigated in preclinical and clinical settings [1]. Periodontal ligament stem cells (PDLSCs) are also multipotent differentiative progenitor cells [2]. For clinical use of progenitor cells, improving the efficacy of homing and engraftment of progenitor cells to damaged tissues is to be one of the beneficial strategies in stem cell-based therapy [3,4]. Controlling the potential of progenitor cells to proliferate and differentiate will be also a critical factor for the therapy.

Considerable evidence has proven that the migration of progenitor cells was regulated by numerous factors and their specific receptors [5]. Angiopoietin 1 (Ang1), an important factor for endothelial survival and proliferation, generates stable and functional vasculature via interaction with Tie-1 and Tie-2 receptors [6]. There is a report showing the new role of Ang-1 as a cell primer [7], where primed progenitor cells increased the expression of adhesion molecules and commitment to endothelial lineage leading to improved engraftment into ischemic tissue. Especially, recent studies have highlighted the potential of COMP-Ang1, a recombinant chimeric protein of Ang1 with a short coiled-coil domain from cartilage oligomeric matrix protein (COMP). COMP-Ang1 has a more stable angiogenic activity than does native Ang1 in vascular formation, and lacks disadvantages such as aggregation and insolubility [8]. COMP-Ang1 appeared to exert beneficial effects in diabetic [9] and ischemic injuries [10]. COMP-Ang1 also enhanced bone morphogenetic protein (BMP)-2-stimulated osteoblast differentiation [11] and accelerated bone formation with increased angiogenesis [12]. These reports suggested that stimulating progenitor cells with COMP-Ang1 may improve their

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therapeutic potentials in a clinical use. However, the effects of COMP-Ang1 on proliferation, differentiation, and migration of progenitor cells, especially of BMMSCs and PDLSCs, and the associated mechanisms are still unclear.

It is suggested that p38 mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/Akt pathways regulate stromal cell-derived factor-1/CXCR4-mediated cell migration as well as survival and differentiation in various cell types [13,14]. We first investigated the effects of COMP-Ang1 on proliferation, differentiation, and migration of BMMSCs and PDLSCs. We also explored the functional roles of p38 MAPK and PI3K/Akt pathways on the cellular events in COMP-Ang1-stimulated cells. Further, we examined the effect of COMP-Ang1 on migration of PDLSCs using a calvarial bone defect animal. Our results suggested that stimulation of progenitor cells with COMP-Ang1 increased their potentials to differentiate and migrate, where the elevated induction of Tie-2 with concomitant activation p38 MAPK, PI3K/Akt pathway, or both was involved.

2. Materials and methods

2.1. Chemicals, laboratory wares, and animals

Carrier-free recombinant COMP-Ang1 and fetal bovine serum (FBS) was purchased from Enzo Life Sciences Inc. (Farmingdale, NY) and Gibco-BRL (Gaithersburg, MD), respectively. Unless specified otherwise, all chemicals and laboratory wares were obtained from Sigma–Aldrich Co. (St. Louis, MO) and Falcon Labware (Becton–Dickinson, Franklin Lakes, NJ), respectively. Male Sprague–Dawley rats (four weeks old) were obtained from Orient Bio (Daejeon, South Korea). The animal care and use in this study were approved by the Chonbuk National University Committee on Ethics in the Care and Use of Laboratory Animals (CBU 2012-0039).

2.2. Cell cultures

BMMSCs were collected from tibia and femoral bones of male Sprague–Dawley rats (four week-old, $n = 10$) and cultured in MesenPRO RS™ basal medium supplemented with MesenPRO RS™ growth supplement (Gibco, Carlsbad, CA) and antibiotics (Gibco), as described previously [15]. Calvarial bones were also collected from the rats used for collection of BM and primary osteoblasts were isolated. PDLSCs were obtained from seven healthy men aged 18–25 years (mean age: 21.2), who underwent molar extraction, and cultured by the methods as described previously [2]. Written informed consent for use of periodontal tissue was obtained from all donors. This study was approved by the Institutional Review Board of Chonbuk National University Hospital.

2.3. Induction of osteoblast differentiation

When cells reached 70–80% confluence, the culture media was changed to osteogenic medium containing 10% FBS and DAG

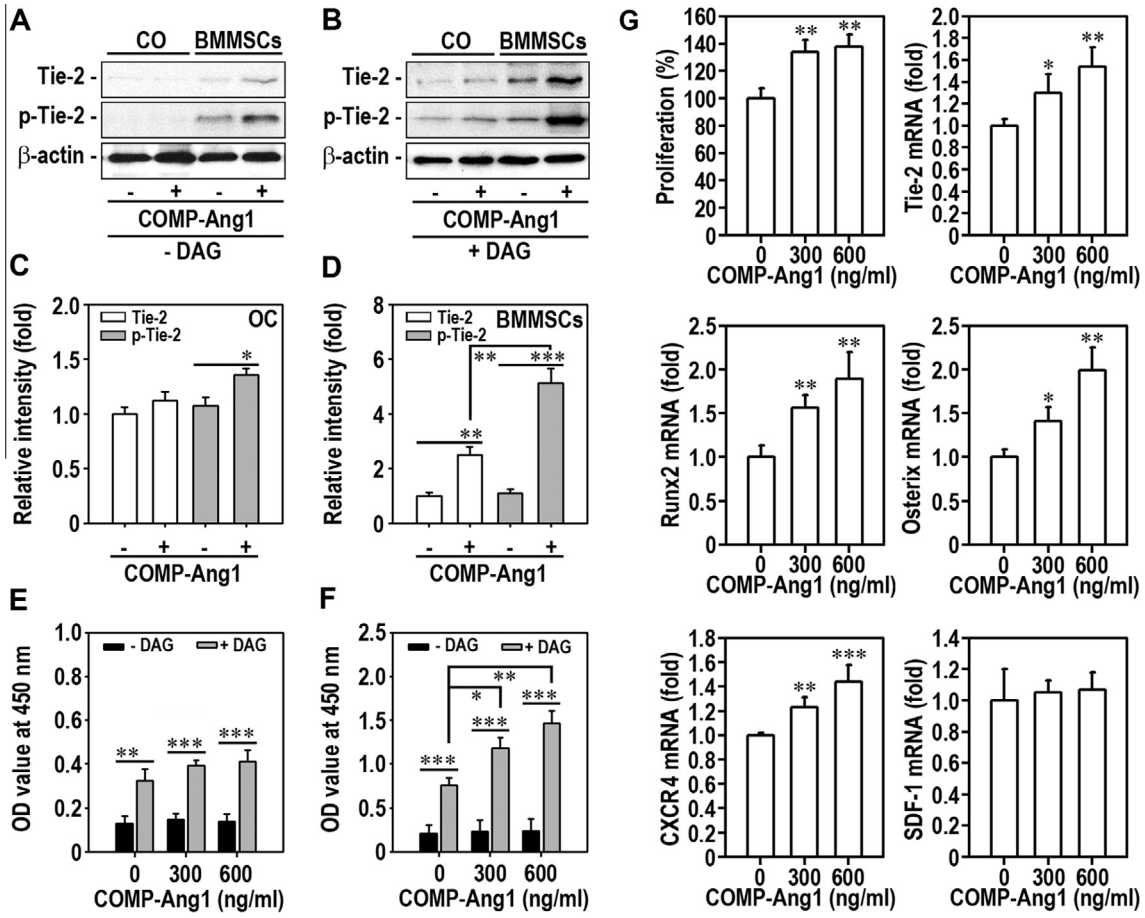

Fig. 1. Different Tie-2 induction and cellular response to COMP-Ang1 according to cell type. Primary calvarial osteoblasts and BMMSCs were treated with COMP-Ang1 in the absence (A) or presence (B) of DAG for three days followed by Western blot analysis. C and D show relative intensities of Tie-2 and p-Tie-2 proteins induced in osteoblasts and BMMSCs, respectively. (E) Osteoblasts and (F) BMMSCs were incubated in the presence of DAG with the indicated concentrations of COMP-Ang1. After 14 days of incubation, the absorbance of alizarin red dye was measured at 450 nm. (G) BMMSCs were exposed to the indicated concentrations of COMP-Ang1 for three days and then processed for real time RT-PCR analysis. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ *vs.* the values between the indicated experiments or the untreated control values.

(100 nM dexamethasone, 50 μM ascorbic acid, and 5 mM β-glycerophosphate) in α-minimum essential medium. These cells were exposed to various doses of COMP-Ang1 (0–600 ng/ml) in the presence or absence of pharmacological inhibitors specific for p38 MAPK (20 μM SB203580) and PI3K (20 μM LY294002). Culture medium was changed once every three days during the experimental periods during the course of incubation (0–14 days).

2.4. Small interfering (si) RNA transfection

BMMSCs and PDLSCs were spread onto 60-mm culture dishes (4×10^5 cells per dish). When these cells reached 70–80% confluence, the cultures were transfected with 50 nM Tie-2 siRNA or scrambled siRNA oligonucleotides using DharmaFECT 1 siRNA Transfection Reagent (Dharmacon, Lafayette, CO) according to manufacturer's instructions. After 24 h of transfection, cells were treated with DAG and/or COMP-Ang1.

2.5. Assays for proliferation and mineralization

Proliferation rates of cells were measured using the Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's instructions. The degree of mineralization was determined by measuring absorbance of the dye at 450 nm after alizarin red staining as described previously [16]. Alkaline phosphatase (ALP) activity was measured as described previously [16]. Type I collagen (COLI) content was determined by a Sirius Red-based colorimetric assay, while osteocalcin (OCN) content was measured using a sandwich ELISA assay kit (Biomedical Technologies Inc., Stoughton, MA), as described previously [17].

2.6. Real time RT-PCR

Total RNA was prepared from cells using Trizol reagent (Invitrogen Corp., Carlsbad, CA). RNA (1 μg) was utilized for cDNA synthesis

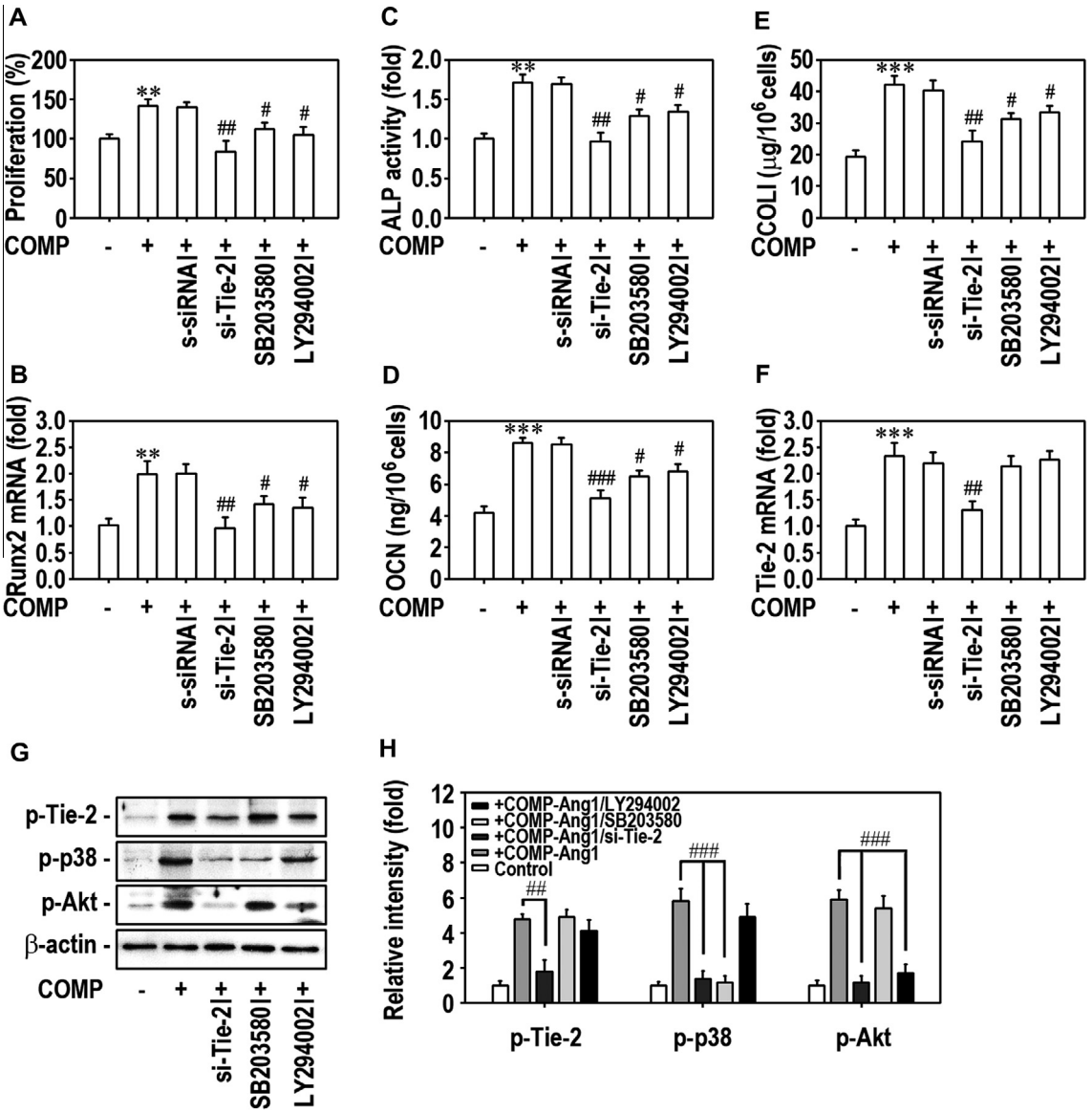

Fig. 2. Signaling pathways involved in COMP-Ang1-stimulated proliferation and osteoblast differentiation of BMMSCs. BMMSCs were incubated in DAG-containing medium with 600 ng/ml COMP-Ang1, 20 μM SB203580, and/or 20 μM LY294002. A portion of these cells was transfected with scrambled or Tie-2-specific siRNA 24 h before supplementation with DAG and/or COMP-Ang1. After various times (3–10 days) of incubation, proliferation (A), *Runx2* expression (B), ALP activity (C), the levels of OCN (D) and COLI (E), and *Tie-2* level (F) were measured. (G) BMMSCs were also incubated with DAG for 24 h and then stimulated with 600 ng/ml COMP-Ang1 for 1 h before immunoblotting. (H) Relative intensities of the protein levels were calculated after normalizing band intensities to β-actin. $^{**}p < 0.01$ and $^{***}p < 0.001$ *vs.* DAG treatment alone. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ *vs.* COMP-Ang1 only.

with SuperScript Reverse Transcriptase II and oligo dT_{12-18} primers (Invitrogen). Oligonucleotide primers for PCR were designed with product sizes less than 200 bp using primer express 3.0 (Applied Biosystems, Foster City, CA) as shown in Supplement Table 1. Power SYBR Green PCR Master Mix was used to detect the accumulation of PCR product during cycling with the ABI StepOnePlus sequence detection system. The thermocycling conditions were as follows: predenaturation at 95 °C for 10 min, and amplification using three-step cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s, for 40 cycles.

2.7. Western blot analysis

Cell lysates were prepared in an NP-40 lysis buffer. Equal amounts of protein extracts were separated by 10–12% SDS–PAGE and blotted onto polyvinylidene difluoride membranes. The blots were probed with primary antibodies overnight at 4 °C prior to incubation with secondary antibody in a blocking buffer for 1 h. The blots were developed with enhanced chemiluminescence and exposed to X-ray film.

2.8. Transwell migration assay

BMMSCs and PDLSCs were cultured in a MSC growth medium bullet kit™ solution (Lonza, Basel, Switzerland) in 60-mm culture dishes in the presence and absence of 3 mg/ml AMD3100 (CXCR4-specific peptide antagonist), 20 μM LY294002, or 20 μM SB203580. Thereafter, 5×10^5 cells were divided onto each well of the upper chamber of 24-transwell plates (8-μm pore size) in the presence and absence of 600 ng/ml COMP-Ang1 on the bottom of the plates. Cells were incubated for additional 12 h at 37 °C and then the numbers of cells migrated into the lower chamber were collected and counted using a hemocytometer. In addition, equal numbers of the migrated cells were resuspended in a rat MSC osteogenic SingleQuots™ kit solution and then spread onto 24-well culture plates (10^5 cells per well). After two weeks of incubation, cells were stained with alizarin red S and absorbance of the dye was measured at 450 nm.

2.9. In vivo migration assay

Male Sprague–Dawley rats ($n = 18$) weighting 250–300 g were used for surgery. The surgical procedures to produce critical-sized calvarial defects were performed according to methods described elsewhere [18]. After anesthesia, animals were divided into three groups (absorbable collagen sponge (ACS) only, $n = 6$; ACS with PDLSCs, $n = 6$; and ACS with PDLSCs and COMP-Ang1, $n = 6$). In this study, ACSs in 8 mm diameter were prepared according to the methods described elsewhere [19]. For all groups, a circular bone defect (8 mm in diameter) was created in the calvarias of rats using a trephine burr without dural perforation. The defects were filled with ACS containing PBS or 10 μg COMP-Ang1 and the surgical sites were carefully sutured. After 24 h of suture, PDLSCs (5×10^6 -cells) labeled with a CM-Dil (Molecular Probes, Carlsbad, CA) were resuspended in PBS and transplanted into rats using a tail vein injection. Two weeks after the injection, ACSs were removed from rats and stained with human CD90 and 4′,6-diamidino-2-phenylindole (DAPI). The homing of PDLSCs into the defect area was quantified either by calculating the ratio (%) of Dil-positive stained cells to DAPI positive cells after counting 500 cells per each ACS or by measuring CD90-specific fluorescence intensity.

2.10. Statistical analysis

All data are expressed as the mean ± standard error (SE) from at least five different samples. A one-way analysis of variance (ANOVA) followed by Scheffe's test was used for multiple comparisons using the SPSS program (version 12.0). Student *t*-test was also used to determine significance differences between two sets of data. A value of $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Different Tie-2 induction and cellular responses to COMP-Ang1 according to cell type

COMP-Ang1-stimulated bone formation appears to correlate with increased angiogenesis [9–12]. COMP-Ang1 may affect differently cellular responses according to cell type [11]. This difference can be coupled with potential or property of cells capable of inducing Tie-2. In this study, the endogenous levels of Tie-2 and p-Tie-2 were found to be detectable in BMMSCs, and were augmented by treating with 600 ng/ml COMP-Ang1, while these proteins were not detectable in calvarial osteoblasts, even in the presence of COMP-Ang1 (Fig. 1A). Primary osteoblasts had detectable Tie-2 and p-Tie-2 levels, as well as a mild increase in p-Tie-2 level ($p < 0.05$) in the presence of COMP-Ang1, when those cells were incubated in medium containing DAG for the same time (Fig. 1B and C). In the presence of DAG, however, BMMSCs exhibited a marked induction of Tie-2 and p-Tie-2 proteins (Fig. 1B), and a COMP-Ang1-mediated significant increase (2.24-fold, $p < 0.01$) in the ratio of p-Tie-2 to total Tie-2 (Fig. 1D). Both BMMSCs ($p < 0.001$) and primary osteoblasts ($p < 0.01$) showed a significant increase in optical density values, corresponding to alizarin red dye staining, according to supplementation with DAG (Fig. 1E and F). In contrast, BMMSCs only showed a dose-dependent elevation in absorbance by COMP-Ang1 (Fig. 1F). COMP-Ang1 also stimulated proliferation of BMMSCs and increased the expression of *Tie-2*,

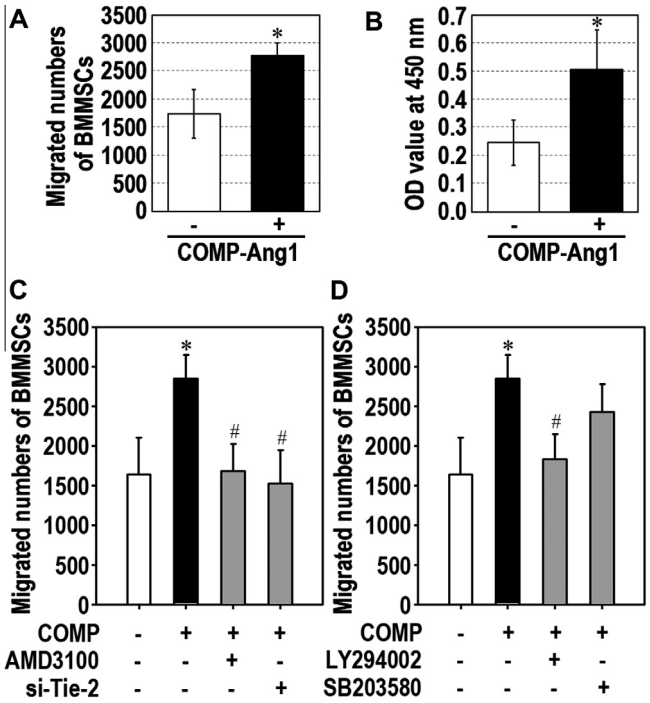

Fig. 3. COMP-Ang1-stimulated migration of BMMSCs *via* activation of Tie-2-mediated PI3K/Akt pathway. (A) BMMSCs were incubated in 24-transwell plates with 600 ng/ml COMP-Ang1, and after 12 h of incubation, migrated cells were counted. (B) Migrated cells were cultured in osteogenic medium for 14 days and then stained with alizarin red. (C and D) BMMSCs or the cells transfected with si-Tie-2 were treated with 600 ng/ml COMP-Ang1, 3 mg/ml AMD3100, 20 μM LY294002, or 20 μM SB203580 for 12 h and then the migrated cells were counted. $^*p < 0.05$ *vs.* the untreated control. $^\#p < 0.05$ *vs.* the COMP-Ang1 only.

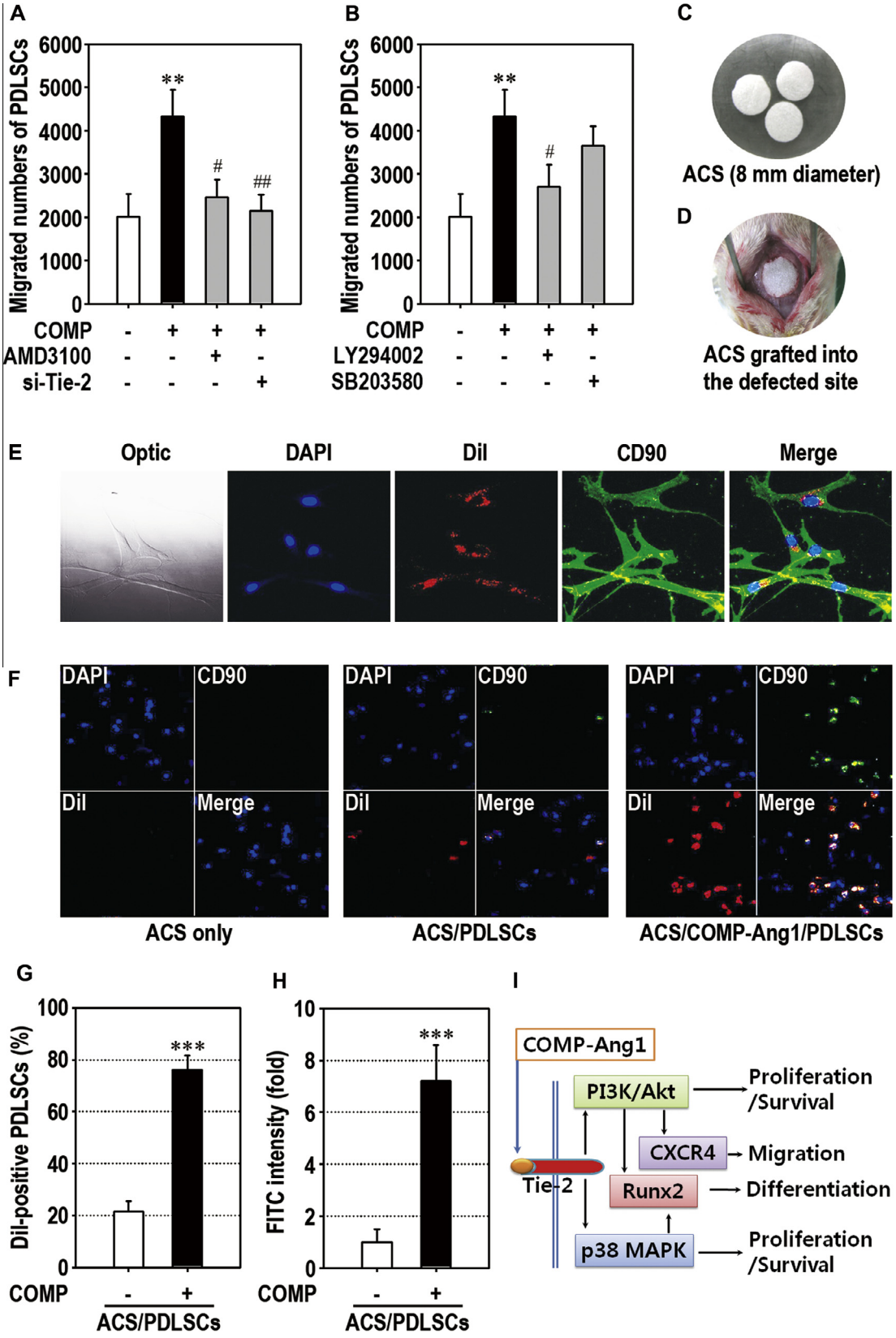

Fig. 4. COMP-Ang1 stimulates *in vitro* and *in vivo* migration of PDLSCs. (A and B) PDLSCs or the cells transfected with si-Tie-2 were incubated in 24-transwell plates with COMP-Ang1 and/or pharmacological inhibitors for 12 h and then the migrated cells were counted. $^{**}p < 0.01$ *vs.* the untreated control. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs.* the COMP-Ang1 only. C and D show ACSs used and implantation of ACS into the calvarial defect of rats, respectively. (E) Confocal microscopic observation of PDLSCs stained with Dil, CD90, and DAPI, where CD90 was used as marker for the cells. (F) Dil-labeled PDLSCs were injected into rats and after two weeks, ACSs were removed and stained with DAPI and CD90. (G) Dil-positive cells (%) and (H) FITC intensity (fold) were calculated from the ACS + PDLSC groups with and without COMP-Ang1. (I) Scheme for the potential of COMP-Ang1 to stimulate cellular responses and the associated mechanisms therein.

runt-related transcription factor 2 (Runx2), *osterix*, and *CXCR4* (Fig. 1G). Furthermore, DAG-mediated increases in expression of osteoblast-specific markers and CXCR4, ALP activity, and calcium accumulation were significantly ($p < 0.05$) augmented by COMP-Ang1 in PDLSCs (Supplement Fig. 1A–D).

Tie-2 translocalization is the main event in COMP-Ang1-stimulated angiogenesis and vascular enlargement [8,20]. The Tie-2 receptor, a specific target of COMP-Ang1, is expressed in quiescent BM hematopoietic stem cells, but is not detected in primary osteoblasts or MC3T3-E1 cells [11,15]. Our current findings support that native expression of Tie-2 differs among cell types, and that its induction is an important event required for COMP-Ang1-mediated stimulation of proliferation, osteogenesis, and angiogenesis. Taken together, we consider that COMP-Ang1 may stimulate progenitor cells more directly than primary osteoblasts, which is due to the different induction of Tie-2.

3.2. Akt and p38 MAPK signaling pathways are involved in COMP-Ang1-stimulated proliferation and differentiation of BMMSCs

Ang1/Tie-2 signaling pathway is considered to regulate proliferation and osteogenic differentiation of MSCs through the activation of p38 MAPK and Akt pathways [15]. We examined the roles of these signaling molecules by transfecting BMMSCs with si-Tie-2 or by treating them with p38 MAPK and Akt inhibitors. In this experiment, cells were treated with DAG 24 h before exposure to 600 ng/ml COMP-Ang1. As shown in Fig. 2A, COMP-Ang1 increased proliferation of BMMSCs and this was significantly inhibited by *Tie-2* knockdown ($p < 0.01$) and by treating SB203580 ($p < 0.05$) or LY294002 ($p < 0.05$). The addition of COMP-Ang1 also augmented *Runx2* expression (Fig. 2B), ALP activity (Fig. 2C), and the levels of OCN (Fig. 2D) and COLI (Fig. 2E). The augmented expressions were diminished by knockdown of *Tie*-2 gene ($p < 0.01$) and by supplementation with SB203580 ($p < 0.05$) or LY294002 ($p < 0.05$). An approximately 2.3-fold increase of *Tie-2* level was induced by COMP-Ang1, whereas this increase was not blocked by the p38 MAPK and PI3K inhibitors (Fig. 2F). Similarly, COMP-Ang1-mediated phosphorylation of Tie-2 was prevented by *Tie-2* knockdown, but not either by SB203580 or LY294002 (Fig. 2G and H). Knockdown of *Tie-2* diminished almost completely ($p < 0.001$) COMP-Ang1-mediated increases in the levels of p-p38 MAPK and p-Akt (Fig. 2H). COMP-Ang1-stimulated phosphorylation of Tie-2, p38 MAPK, and Akt and their suppression by knockdown of *Tie-2* were also found in PDLSCs (data not shown).

Based on previous studies, Tie-2-mediated activation of p38 MAPK and Akt signaling pathways is a possible mechanism involved in COMP-Ang1-stimulated proliferation and osteogenesis in MSCs. Our present findings strongly support the role of Tie-2 in activating p38 MAPK and Akt as the upstream effector. There are other reports that p38 MAPK and Akt signaling pathways are involved in differentiation of MSCs [21]. Tie-2 phosphorylation followed by Ang1 binding also activated the p38 MAPK and Akt signaling pathways in endothelial cells [22]. Our current data, along with previous findings, suggest that Tie-2-induced activation of the p38 MAPK and Akt signaling pathways is involved in COMP-Ang1-stimulated proliferation and osteogenesis. As Akt promotes osteoblast differentiation induced by BMP2 and enhances the function, transcriptional activity, and stability of Runx2 [23], we also consider that Tie-2-PI3K/Akt pathway may involve osteoblast differentiation through activation of Runx2.

3.3. Tie-2-induced PI3K/Akt pathway is involved in COMP-Ang1-stimulated migration of BMMSCs and PDLSCs

COMP-Ang1 significantly stimulated migration of BMMSCs (Fig. 3A). The number of alizarin red-positive cells was also higher in COMP-Ang1-exposed cells than in non-treated control cells (Fig. 3B). The expression of CXCR4 was observed on the surface of MSCs and several tissue committed stem/progenitor cells [24]. It has been reported that a small proportion of MSCs express CXCR4, which contributes to their migration *in vitro* [25]. Therefore, we investigated the mechanisms involved in COMP-Ang1-stimulated migration of the cells by treating AMD3100 or by transfecting si-Tie-2 (Fig. 3C). Both AMD3100 treatment and *Tie-2* knockdown significantly ($p < 0.05$) inhibited the migration stimulated by COMP-Ang1, suggesting that COMP-Ang1-induced BMMSC migration was mainly due to its receptor CXCR4 that was induced by Tie-2 pathway. Pretreating the cells with LY294002, but not with SB203580, also suppressed COMP-Ang1-stimulated migration of the cells (Fig. 3D).

Consistent with the results from BMMSCs, COMP-Ang1 significantly ($p < 0.01$) increased migration of PDLSCs and this increase was attenuated by AMD3100 or LY294002 treatment, or by *Tie-2* knockdown (Fig. 4A and B). Although p38 MAPK inhibitor appeared to diminish migration of the cells, there was no a significant difference (Fig. 4B). We subsequently examined the effect of COMP-Ang1 on *in vivo* migration of PDLSCs. Fig. 4C and D shows ACSs applied and the implantation of ACS into the calvarial defects, respectively. We checked the characteristic of PDLSCs by staining with DAPI (blue), Dil (red), and CD90 (green) before transplantation (Fig. 4E). When ACSs were removed and stained with CD90 and DAPI, there were no signals specific for Dil and CD90 in the ACS only group (Fig. 4F). However, greater fluorescent intensities specific for Dil and CD90 were observed in the group combined with PDLSCs and COMP-Ang1 than that only with PDLSC injection (Fig. 4F). Combined treatment with COMP-Ang1 and PDLSCs also revealed higher levels in Dil-positive PDLSCs ($p < 0.001$, Fig. 4G) and in FITC intensity ($p < 0.001$, Fig. 4H), compared with the group injected with PDLSC only. These results supported the potential of COMP-Ang1 to stimulate migration of progenitor cells. Taken as a whole, our results demonstrate that COMP-Ang1 activates PI3K/Akt and p38 MAPK signal transduction pathways through induction of Tie-2, which stimulates proliferation, osteoblast differentiation, or migration of BMMSCs and PDLSCs (Fig. 4I). Further studies to clarify the cellular mechanisms of *in vivo* migration by COMP-Ang1 and to explore whether the migrated progenitor cells participate in healing of damaged tissues will be needed.

Acknowledgment

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.11.025.

References

[1] F.P. Barry, J.M. Murphy, Mesenchymal stem cells: clinical applications and biological characterization, Int. J. Biochem. Cell Biol. 36 (2004) 568–584.
[2] S.Y. Kim, J.Y. Lee, Y.D. Park, K.L. Kang, J.C. Lee, J.S. Heo, Hesperetin alleviates the inhibitory effects of high glucose on the osteoblastic differentiation of periodontal ligament stem cells, PLoS ONE 8 (2013) e67504.
[3] G. Vogel, Cell biology. Stem cells: new excitement, persistent questions, Science 290 (2000) 1672–1674.
[4] A.J. Wagers, I.L. Weissman, Plasticity of adult stem cells, Cell 116 (2004) 639–648.
[5] H. Ito, Chemokines in mesenchymal stem cell therapy for bone repair: a novel concept of recruiting mesenchymal stem cells and the possible cell sources, Mod. Rheumatol. 21 (2011) 113–121.

[6] C. Suri, P.F. Jones, S. Patan, S. Bartunkova, P.C. Maisonpierre, S. Davis, T.N. Sato, G.D. Yancopoulos, Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis, Cell 87 (1996) 1171–1180.
[7] M.S. Kim, C.S. Lee, J. Hur, H.J. Cho, S.I. Jun, T.Y. Kim, S.W. Lee, J.W. Suh, K.W. Park, H.Y. Lee, H.J. Kang, D.S. Lee, G.Y. Koh, H. Nakagami, R. Morishita, Y.B. Park, H.S. Kim, Priming with angiopoietin-1 augments vasculogenic potential of the peripheral blood stem cells mobilized with G-CSF through novel Tie2/Ets-1 pathway, Circulation 120 (2009) 2240–2250.
[8] C.H. Cho, R.A. Kammerer, H.J. Lee, M.O. Steinmetz, Y.S. Ryu, S.H. Lee, K. Yasunaga, K.T. Kim, I. Kim, H.H. Choi, W. Kim, S.H. Kim, S.K. Park, G.M. Lee, G.Y. Koh, COMP-Ang1: a designed angiopoietin-1 variant with nonleaky angiogenic activity, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 5547–5552.
[9] C.H. Cho, H.K. Sung, K.T. Kim, H.G. Cheon, G.T. Oh, H.J. Hong, O.J. Yoo, G.Y. Koh, COMP-angiopoietin-1 promotes wound healing through enhanced angiogenesis, lymphangiogenesis, and blood flow in a diabetic mouse model, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 4946–4951.
[10] H.Y. Shin, Y.J. Lee, H.J. Kim, C.K. Park, J.H. Kim, K.C. Wang, D.G. Kim, G.Y. Koh, S.H. Paek, Protective role of COMP-Ang1 in ischemic rat brain, J. Neurosci. Res. 88 (2010) 1052–1063.
[11] B.C. Jeong, H.J. Kim, I.H. Bae, K.N. Lee, K.Y. Lee, W.M. Oh, S.H. Kim, I.C. Kang, S.E. Lee, G.Y. Koh, K.K. Kim, J.T. Koh, COMP-Ang1, a chimeric form of angiopoietin 1, enhances BMP2-induced osteoblast differentiation and bone formation, Bone 46 (2010) 479–486.
[12] B.H. Park, S.J. Yoon, K.Y. Jang, M.R. Kim, H.S. Lee, K.B. Kim, H. Park, S.Y. Lee, H.S. Park, S.T. Lim, K.J. Song, J.R. Kim, COMP-angiopoietin-1 accelerates bone formation during distraction osteogenesis, Bone 46 (2010) 1442–1448.
[13] S.B. Peng, V. Peek, Y. Zhai, D.C. Paul, Q. Lou, X. Xia, T. Eessalu, W. Kohn, S. Tang, Akt activation, but not extracellular signal-regulated kinase activation, is required for SDF-1alpha/CXCR4-mediated migration of epithelioid carcinoma cells, Mol. Cancer Res. 3 (2005) 227–236.
[14] C.H. Ryu, S.A. Park, S.M. Kim, J.Y. Lim, C.H. Jeong, J.A. Jun, J.H. Oh, S.H. Park, W.I. Oh, S.S. Jeun, Migration of human umbilical cord blood mesenchymal stem cells mediated by stromal cell-derived factor-1/CXCR4 axis via Akt, ERK, and p38 signal transduction pathways, Biochem. Biophys. Res. Commun. 398 (2010) 105–110.
[15] S. Kim, J.C. Lee, E.S. Cho, J. Kwon, COMP-Ang1 promotes chondrogenic and osteogenic differentiation of multipotent mesenchymal stem cells through the Ang1/Tie2 signaling pathway, J. Orthop. Res. 31 (2013) 1920–1928.
[16] S.H. Kook, Y.M. Jeon, S.S. Park, J.C. Lee, Periodontal fibroblasts modulate proliferation and osteogenic differentiation of embryonic stem cells through production of fibroblast growth factors, J. Periodontol. 85 (2014) 645–654.
[17] Y.M. Jeon, S.H. Kook, S.J. Rho, S.S. Lim, K.C. Choi, H.S. Kim, J.G. Kim, J.C. Lee, Fibroblast growth factor-7 facilitates osteogenic differentiation of embryonic stem cells through the activation of ERK/Runx2 signaling, Mol. Cell. Biochem. 382 (2013) 37–45.
[18] J.H. Jung, J.H. Yun, Y.J. Um, U.W. Jung, C.S. Kim, S.H. Choi, K.S. Cho, Bone formation of *Escherichia coli* expressed rhBMP-2 on absorbable collagen block in rat calvarial defects, Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 111 (2011) 298–305.
[19] S.T. Boyce, D.J. Christianson, J.F. Hansbrough, Structure of a collagen-GAG dermal skin substitute optimized for cultured human epidermal keratinocytes, J. Biomed. Mater. Res. 22 (1988) 939–957.
[20] C.H. Cho, K.E. Kim, J. Byun, H.S. Jang, D.K. Kim, P. Baluk, F. Baffert, G.M. Lee, N. Mochizuki, J. Kim, B.H. Jeon, D.M. McDonald, G.Y. Koh, Long-term and sustained COMP-Ang1 induces long-lasting vascular enlargement and enhanced blood flow, Circ. Res. 97 (2005) 86–94.
[21] Y. Wang, B.R. Weil, J.L. Herrmann, A.M. Abarbanell, J. Tan, T.A. Markel, M.L. Kelly, D.R. Meldrum, MEK, p38, and PI-3K mediate cross talk between EGFR and TNFR in enhancing hepatocyte growth factor production from human mesenchymal stem cells, Am. J. Physiol. Cell Physiol. 297 (2009) C1284–C1293.
[22] C. Schliemann, R. Bieker, T. Padro, T. Kessler, H. Hintelmann, T. Buchner, W.E. Berdel, R.M. Mesters, Expression of angiopoietins and their receptor Tie2 in the bone marrow of patients with acute myeloid leukemia, Haematologica 91 (2006) 1203–1211.
[23] Y.H. Choi, Y.J. Kim, H.M. Jeong, Y.H. Jin, C.Y. Yeo, K.Y. Lee, Akt enhances Runx2 protein stability by regulating Smurf2 function during osteoblast differentiation, FEBS J. 281 (2014) 3656–3666.
[24] A. Pituch-Noworolska, M. Majka, A. Janowska-Wieczorek, M. Baj-Krzyworzeka, B. Urbanowicz, E. Malec, M.Z. Ratajczak, Circulating CXCR4-positive stem/progenitor cells compete for SDF-1-positive niches in bone marrow, muscle and neural tissues: an alternative hypothesis to stem cell plasticity, Folia Histochem. Cytobiol. 41 (2003) 13–21.
[25] M. Shi, J. Li, L. Liao, B. Chen, B. Li, L. Chen, H. Jia, R.C. Zhao, Regulation of CXCR4 expression in human mesenchymal stem cells by cytokine treatment: role in homing efficiency in NOD/SCID mice, Haematologica 92 (2007) 897–904.