Molecules and Cells

The Significance of SDF-1α-CXCR4 Axis in *in vivo* Angiogenic Ability of Human Periodontal Ligament Stem Cells

Yoon-Kyung Bae[1,2,3,9], Gee-Hye Kim[4,9], Jae Cheoun Lee[5], Byoung-Moo Seo[6], Kyeung-Min Joo[1,2,3,7], and Gene Lee[4,*], and Hyun Nam[2,3,8,*]

[1]Department of Health Sciences and Technology, SAIHST, Sungkyunkwan University, Seoul 06351, Korea, [2]Single Cell Network Research Center, Sungkyunkwan University School of Medicine, Suwon 16419, Korea, [3]Stem Cell and Regenerative Medicine Center, Research Institute for Future Medicine, Samsung Medical Center, Seoul 06351, Korea, [4]Laboratory of Molecular Genetics, Dental Research Institute, School of Dentistry, Seoul National University, Seoul 03080, Korea, [5]Children's Dental Center and CDC Baby Tooth Stem Cell Bank, Seoul 06072, Korea, [6]Department of Oral and Maxillofacial Surgery, School of Dentistry, Seoul National University, Seoul 03080, Korea, [7]Department of Anatomy & Cell Biology, Sungkyunkwan University School of Medicine, Suwon 16419, Korea, [8]Department of Neurosurgery, Samsung Medical Center, Sungkyunkwan University, Seoul 06351, Korea, [9]These authors contributed equally to this work.
*Correspondence: genelee@snu.ac.kr (GL); snutaeng@gmail.com (HN)
http://dx.doi.org/10.14348/molcells.2017.0004
www.molcells.org

Periodontal ligament stem cells (PDLSCs) are multipotent stem cells derived from periodontium and have mesenchymal stem cell (MSC)-like characteristics. Recently, the perivascular region was recognized as the developmental origin of MSCs, which suggests the *in vivo* angiogenic potential of PDLSCs. In this study, we investigated whether PDLSCs could be a potential source of perivascular cells, which could contribute to *in vivo* angiogenesis. PDLSCs exhibited typical MSC-like characteristics such as the expression pattern of surface markers (CD29, CD44, CD73, and CD105) and differentiation potentials (osteogenic and adipogenic differentiation). Moreover, PDLSCs expressed perivascular cell markers such as NG2, α-smooth muscle actin, platelet-derived growth factor receptor β, and CD146. We conducted an *in vivo* Matrigel plug assay to confirm the *in vivo* angiogenic potential of PDLSCs. We could not observe significant vessel-like structures with PDLSCs alone or human umbilical vein endothelial cells (HUVECs) alone at day 7 after injection. However, when PDLSCs and HUVECs were co-injected, there were vessel-like structures containing red blood cells in the lumens, which suggested that anastomosis occurred between newly formed vessels and host circulatory system. To block the SDF-1α and CXCR4 axis between PDLSCs and HUVECs, AMD3100, a CXCR4 antagonist, was added into the Matrigel plug. After day 3 and day 7 after injection, there were no significant vessel-like structures. In conclusion, we demonstrated the perivascular characteristics of PDLSCs and their contribution to *in vivo* angiogenesis, which might imply potential application of PDLSCs into the neovascularization of tissue engineering and vascular diseases.

Keywords: angiogenesis, mesenchymal stem cells, periodontal ligament stem cells, perivascular cells, SDF-1α-CXCR4 axis

INTRODUCTION

Human teeth contain various types of stem cells and periodontal ligament stem cell (PDLSC) is one of representative stem cells residing in the periodontium (Seo et al., 2004). Functionally, PDLSCs are thought to regenerate damaged periodontium because PDLSCs can make periodontal ligament-like

Received 11 January, 2017; accepted 29 April, 2017; published online 14 June, 2017

eISSN: 0219-1032

and cementum-like structures *in vivo* (Seo et al., 2004). PDLSCs have the expression pattern of specific surface antigens and *in vitro* differentiation potentials, which are similar to typical mesenchymal stem cells (MSCs) (Ivanovski et al., 2006; Nagatomo et al., 2006). Recently, pericyte-like characteristics of PDLSCs have been reported (Iwasaki et al., 2013), which is in accordance with reports suggesting that the origin of MSCs is the perivascular region (Crisan et al., 2008; Corselli et al., 2010).

Tissue engineering requires rapid vascular networks with the recipient's vessels, which provides nutrients and oxygen, as well as discard exhausted materials (Jain et al., 2005). To solve the problem of vascularization, several solutions including the delivery of angiogenic factors have been suggested. However, there are limitations involved in the vascularization of thick engineered tissues (Isner and Asahara, 1999; Isner et al., 1996). Recently, it was suggested that co-injection of endothelial (progenitor) cells and perivascular cells could result in the formation of microvessels *in vivo*, which might be useful for tissue engineering (Au et al., 2008; Melero-Martin et al., 2007).

The stromal cell-derived factor 1 (SDF-1) is one of CXC chemokines via binding to CXCR4 for signal transduction (Aiuti et al., 1997; Mohle et al., 1998). The SDF-1α and CXCR4 axis is an important signaling pathway in neovascularization, including embryonic vasculogenesis and cancer (Petit et al., 2007; Teicher and Fricker, 2010). Recently, the beneficial roles of SDF-1α were reported in the neovascularization in cardiac infarct (Huang et al., 2011; Wang et al., 2012) and regeneration in spinal cord injury, which showed that the upregulated level of SDF-1 at the injury site could recruit stem cells (Jaerve et al., 2012). Moreover, SDF-1 is involve in the migration, survival, proliferation, and differentiation of stem cells (Zemani et al., 2008).

Recently, PDLSCs are reported to have perivascular characteristics (Iwasaki et al., 2013). In this study, we investigated whether PDLSCs could be a potential source of perivascular cells, which contributed to *in vivo* angiogenesis. Primarily isolated PDLSCs from the third molar showed typical MSC-like characteristics including surface antigens and differentiation potentials. To confirm the perivascular characteristics of PDLSCs, the expression of perivascular cell markers was verified by quantitative PCR and FACS analysis. The *in vivo* angiogenesis of PDLSCs was confirmed using *in vivo* Matrigel plug assay, and vessel-like structures could be formed by co-injection of PDLSCs and HUVECs. Finally, the involvement of the SDF-1α-CXCR4 axis between PDLSCs and HUVECs in *in vivo* angiogenesis was determined by CXCR4 antagonist, AMD3100.

MATERIALS AND METHODS

Primary isolation and culture

The usage of human teeth was approved by the Institutional Review Board (S-D20070004). Primary isolation and culture of PDLSCs were conducted according to the previous reports (Lee et al., 2011; Seo et al., 2004). Briefly, periodontal ligament tissues were extracted from human third molars. They were minced and incubated in 1 mg/ml of Collagenase type I (Gibco, USA) and 2.4 mg/ml of Dispase (Gibco) at 37℃ for 1 h. After the inactivation of the enzyme via α-MEM (HyClone, USA) and 10% FBS (HyClone), the cells were washed two times with α-MEM (HyClone). Single-cell suspensions were maintained in α-MEM supplemented with 10% FBS and 1% Antibiotic-Antiomycotic (Gibco). The medium was replaced every 3 days. When cells became 70% confluency, we conducted subculturing with 0.05% Trypsin/EDTA (Gibco). To acquire growth curve, the population doubling length (PDL) was calculated at each passage. Human umbilical vein endothelial cells (HUVECs) were purchased from Lonza and cultured in EGM-2 (Lonza) until the sixth passage. All experiments were conducted using HUVECs from the sixth passage.

FACS analysis

The expression of surface markers was verified using FACS analysis. Cells at third passage were detached and stained with fluorescent-conjugated antibodies, which are listed in Supplementary Table 1. Between 1.0×10^5 and 5.0×10^5 cells were treated with antibodies for 30 min on ice. After washing, FACS data were acquired using a FACS Calibur (Becton Dickinson, USA), which were analyzed using FLOWJO (Tree Star, Inc., USA) software.

In vitro differentiation

Cells were grown to be confluent and cultured in the osteogenic and adipogenic differentiation medium. Osteogenic differentiation medium contained α-MEM and 5% FBS supplemented with 50 μg/ml L-ascorbic acid phosphate (Sigma-Aldrich, USA), 10 mM β-glycerophosphate (Sigma-Aldrich), and 1 μM dexamethasone (Sigma-Aldrich). Adipogenic differentiation medium included α-MEM and 5% FBS supplemented with 10 μg/ml insulin (Sigma-Aldrich), 0.5 mM isobutylmethaylxanthin (Sigma-Aldrich), 50 μM indomethacin (Sigma-Aldrich), and 1 μM dexamethasone. The medium was replaced every 3 days. After 21 days of differentiation, cells were fixed with 10% neutral-buffered formalin at 37℃ for 10 min. After washing, cells were stained with Alizarin red and Oil red O solution (all from Sigma-Aldrich).

Quantitative PCR (qPCR)

The total RNA of PDLSCs and HUVECs was prepared using an RNeasy Mini Kit (Qiagen, USA). For the synthesis of cDNA, 2 μg of total RNA was reverse-transcribed using SuperScript III (Invitrogen TM, USA) according to the manufacture's instruction. qPCR was carried out using each primer and THUNDERBIRD SYBR qPCR Mix (TOYOBO, Japan) in a CFX Connect Real-Time PCR Detection System (Bio-rad, USA). The information of each primer and annealing temperature (Tm) was provided in Supplementary Table 2.

In vivo Matrigel plug assay

Animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) (SNU-1010046). PDLSCs and HUVECs were mixed to be a total of 2.0×10^6 cells at ratios of 1:0, 1:1, and 0:1 in 200 μl of ice-cold Phenol Red-free Matrigel (BD Bioscience, USA). The mixture was injected into 8-week-old immunodeficient mice (NIH-bg-nu-xid, Harlan Sprague-Dawley, USA). A mouse was injected by

one Matrigel implant subcutaneously using a 25-gauge needle. To inhibit the SDF-1α and CXCR4 axis, 10 μM of AMD3100 (Sigma-Aldrich) was added into the Matrigel plug before injection.

Histology

Mice were euthanized at day 3 and day 7 after injection to remove Matrigel plugs. The Matrigel plugs were incubated in 10% neutral-buffered formalin overnight. Slides were prepared and sectioned at 5 μm-thickness. Slides were deparaffinized and rehydrated. Slides were stained with hematoxylin and eosin (all from Sigma-Aldrich), and observed under microscope.

Immunofluorescent staining

Endogenous peroxidase was blocked using 10% hydrogen peroxide (Sigma-Aldrich) for 15 min. After washing, antigen retrieval was performed with pepsin for 20 min at 37℃. After washing, slides were blocked with 10% normal goat serum (Jackson Immunoresearch Lab., USA) for 30 min. The primary antibodies including rabbit anti-CD31 (Santa Cruz Biotechnology, USA) and mouse anti-α-smooth muscle actin (Santa Cruz Biotechnology) were treated to the slides for overnight at 4℃. After washing, secondary antibodies including Alexa 488-conjugated goat-anti mouse IgG (Invitrogen) and Alexa 594-conjugated goat-anti rabbit IgG (Invitrogen) antibodies, were applied to the slides for 1 hour at room temperature. DAPI (Sigma-Aldrich) was used for counterstaining. We observed slides under a Fluoview FV 300 (Olympus, Japan).

RESULTS

Primary isolation and characterization of PDLSCs

Primary isolation of PDLSCs was conducted following a previous report (Lee et al., 2011; Seo et al., 2004). *In vitro* characterization of PDLSCs was conducted at the third passage. PDLSCs had mesenchymal stem cell (MSC)-like bi-polar morphology (Fig. 1A). We did not observe growth regression (Fig. 1B), but at late passages, PDLSCs exhibited a more elongated morphology (data not shown). The expression of surface markers was verified by FACS. PDLSCs expressed MSC markers (CD29, CD44, CD73, CD90, and CD105). However, PDLSCs were negative for hematopoietic cell markers (CD14, CD34, CD45, CD117, and HLA-DR) and the endothelial cell marker CD31 (Fig. 1C). To confirm the differentiation potentials, PDLSCs were cultured to be confluent, and the culture medium was changed to osteogenic or adipogenic medium for 21 days. The results of Alizarin red staining revealed calcium deposits, suggesting osteogenic differentiation of PDLSCs (Fig. 1D). Oil red O stained lipid vacuoles, suggesting adipogenic differentiation of PDLSCs (Fig. 1E).

The perivascular characteristics of PDLSCs

The perivascular region is known to be enriched by stem cells, and it is considered to be the origin of MSCs (Crisan et al., 2008; Corselli et al., 2010). We analyzed the expression of perivascular cells markers in PDLSCs. As shown Fig. 2A, PDLSCs expressed *NG2*, *α-smooth muscle actin (α-SMA)*,

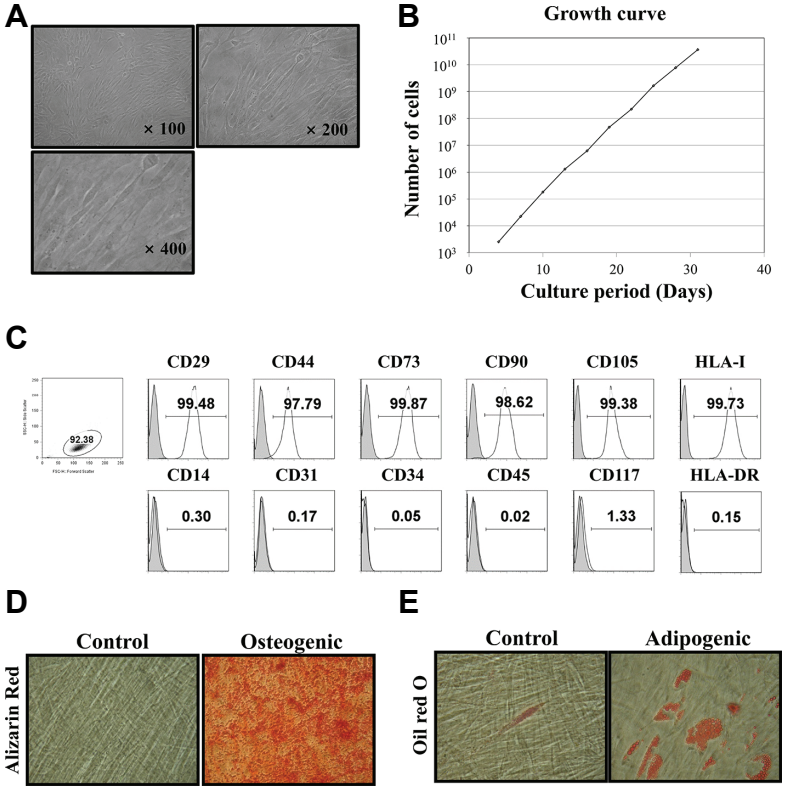

Fig. 1. Primary isolation and characterization of PDLSCs. Primarily isolated PDLSCs were cultured and characterized. (A) PDLSCs showed typical MSC-like morphology at the third passage. (B) The growth of PDLSCs was linear during the culture period. (C) The expression of surface markers was determined by FACS. PDLSCs were positive for MSC markers. PDLSCs were cultured in osteogenic or adipogenic differentiation medium for 21 days. (D, E) Calcium deposits and lipid vacuoles were stained by Alizarin red and Oil red O, respectively.

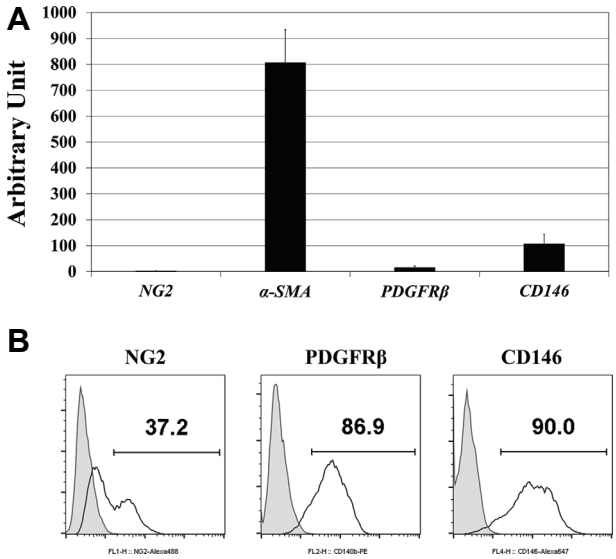

Fig. 2. The expression of perivascular cell markers in PDLSCs. The expression of perivascular cell markers in PDLSCs was determined by qPCR and FACS analysis. (A) The mRNA expression levels of *NG2*, *α-SMA*, *PDGFRβ*, and *CD146* derived from three different lines of PDLSCs were shown as average ± standard deviation. The arbitrary unit on the Y-axis represented $2^{-\Delta CT} \times 10^4$. (B) In the results of FACS analysis, PDLSCs were positive for NG2, PDGFRβ, and CD146 with different expression levels of NG2, PDGFRβ, and CD146. One of representative data was shown.

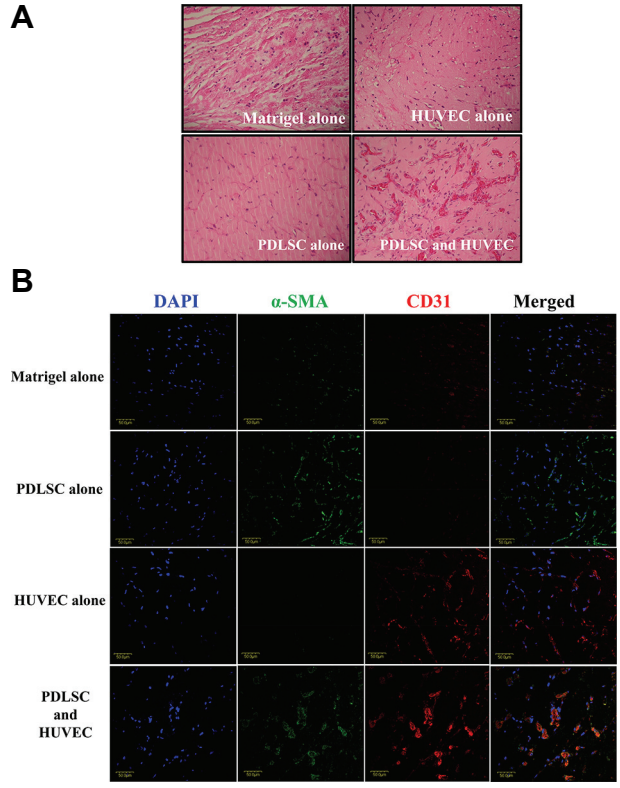

Fig. 3. *In vivo* angiogenic potential of PDLSCs. To investigate the *in vivo* angiogenic potential of PDLSCs, *in vivo* Matrigel plug assay was performed. PDLSCs and HUVECs were subcutaneously injected separately or together into immunodeficient mice. At day 7 after injection, vessel-like structures in Matrigel plug were analyzed by H&E and immunofluorescent staining. (A) In the results of PDLSCs alone or HUVECs alone, there were no obvious vessel-like structures. However, when PDLSCs and HUVECs were co-injected, vessel-like structures were formed and red blood cells were observed in the lumen. (B) Immunofluorescent staining for CD31 and α-SMA revealed that vessel-like structures were formed by α-SMA-positive PDLSCs and CD31-positive HUVECs.

platelet-derived growth factor receptor β (PDGFRβ), and *CD146*. According the results of qPCR, the expression of *α-SMA* was the highest, but that of *NG2* was the lowest. FACS data revealed that PDLSCs expressed NG2, PDGFRβ, and CD146, but the expression level of them was different (Fig. 2B). Despite of the lowest mRNA level of NG2, we could observe the expression of NG2, which showed positive and negative populations. However, the expression of PDGFRβ was homogenous and that of CD146 showed broad range. These data indicated that PDLSCs had perivascular cell-like characteristics, suggesting that the origin of PDLSCs might be the perivascular region. Next, we performed functional involvement of PDLSCs in *in vivo* angiogenesis.

In vivo angiogenic potential of PDLSCs

PDLSCs with or without HUVECs were injected subcutaneously into immunodeficient mice. At day 7 after injection, the Matrigel plug was removed and analyzed by hematoxylin and eosin (H&E) staining. As shown Fig. 3A, in the single injection group of PDLSCs or HUVECs, we could not observe functional vessel-like structures. However, in the co-injection group of PDLSCs and HUVECs, functional vessel-like structures were formed in the Matrigel plug. Moreover, we could observe erythrocytes and leukocytes in the lumen of newly formed vessels, suggestive of anastomosis with host circulatory system. Then, we analyzed the location of injected PDLSCs and HUVECs by staining with CD31 and α-SMA. The expression of α-SMA and CD31 was localized around vessel-like structures, which were formed by PDLSCs and HUVECs (Fig. 3B).

The involvement of SDF-1α and CXCR4 axis in *in vivo* angiogenesis

The SDF-1α-CXCR4 axis is known to regulate neovascularization in development and regeneration (Petit et al., 2007). qPCR data revealed that the expression of SDF-1α was found to be highly upregulated in PDLSCs compared to HUVECs (Fig. 4A). In contrast, the expression of CXCR4 is highly upregulated in HUVECs compared to PDLSCs (Fig. 4A). These contradictory expression patterns suggested reciprocal interactions between PDLSCs and HUVECs. To investigate the contribution of the SDF-1α-CXCR4 axis to *in vivo* angiogenesis, AMD3100, which is a known CXCR4 antagonist, was mixed with PDLSCs and HUVECs in a Matrigel plug

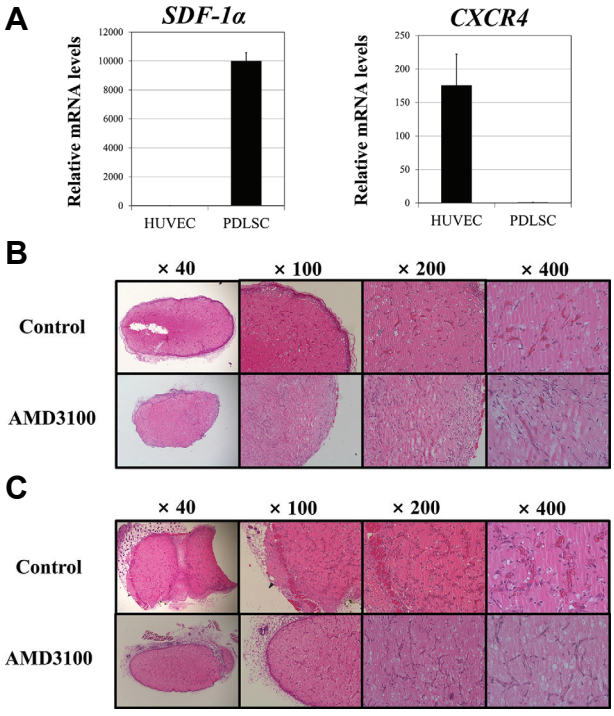

Fig. 4. The involvement of the SDF-1α and CXCR4 axis in *in vivo* angiogenesis by PDLSCs and HUVECs. The expression of SDF-1α and CXCR4 was determined by qPCR. (A) PDLSCs expressed SDF-1α, but CXCR4 expression was not detected. In contrast, HUVECs expressed CXCR4, but SDF-1α expression was not detected. To confirm the functional involvement of the SDF-1α and CXCR4 axis in *in vivo* angiogenesis, AMD3100, a CXCR4 antagonist, was mixed with the Matrigel plug. (B) At day 3 after injection, there were no obvious vessel-like structures in the AMD3100-treated group compared to the control group. (C) At day 7 after injection, there were no vessel-like structures in the AMD3100-treated group compared to the control group.

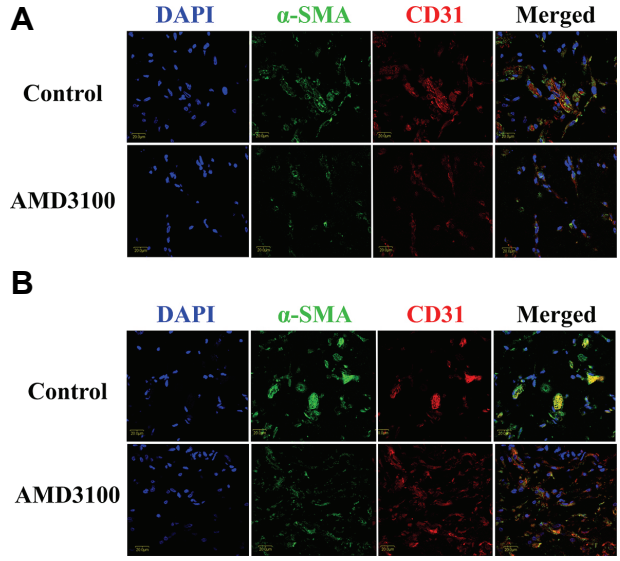

Fig. 5. The effects of blocking the SDF-1α and CXCR4 axis on *in vivo* angiogenesis by PDLSCs and HUVECs. To investigate the localization of PDLSCs and HUVECs, immunofluorescent staining was conducted. Based on the results of immunofluorescent staining for CD31 and α-SMA, we confirmed that vessel-like structures were not formed in the AMD3100-treated group compared to the control group at day 3 and day 7 after injection (A and B, respectively).

assay. At day 3 after injection, in the control group, we observed vessel-like structures, while there were no vessel-like structures in the AMD3100-treated group (Fig. 4B). At day 7 after injection, the size of the vessel-like structures in the control group increased and more red blood cells were observed (Fig. 4C). However, in the AMD3100-treated group, there were no significant vessel-like structures, although small vessel-like structures were observed. Immunofluorescent staining confirmed that the treatment of AMD3100 led to no significant vessel-like structures and anastomosis, and there were no significant differences in this tendency between day 3 and day 7 after injection (Figs. 5A and 5B, respectively).

DISCUSSION

PDLSCs are multipotent stem cells derived from the periodontium that support teeth throughout life. However, despite of the multipotency and beneficial effects of PDLSCs, such as immune modulatory capacity (Wada et al., 2009; 2013), the applications of PDLSCs have been limited to the regeneration of the periodontium and bone formation (Chen and Jin, 2010; Maeda et al., 2011; Zhu and Liang, 2015). In this study, we investigate the feasibility of PDLSCs as a perivascular cell source in tissue engineering and other vascular diseases.

Recently, it was reported that MSCs originate from the perivascular region (Crisan et al., 2008). A previous report suggests that perivascular regions are also the origin of dental pulp stem cells because of the perivascular localization and the expression of perivascular markers (Shi and Gronthos, 2003). Although PDLSCs are isolated from the periodontium, periodontium also contains many vessels, which suggests that PDLSCs may originate from the perivascular region and have pericyte-like characteristics. Although pericyte-specific markers have not been identified, the combined expression of various pericyte markers in PDLSCs suggested that they might have pericyte characteristics and that they might originate from the perivascular region. Recently, the expression of pericyte markers in PDLSCs was reported, and, in this study, we showed that PDLSCs were positive for perivascular cell markers including α-SMA, NG2, PDGFRβ, and CD146. Interestingly, primarily isolated PDLSCs expressed different levels of pericyte markers. These data suggested that PDLSCs are comprised of a heterogeneous population.

Perivascular cells, also called as pericytes are located near endothelial cells and interact reciprocally to regulate development, stabilization, maturation, and remodeling of blood vessels (Armulik et al., 2005; Gaengel et al., 2009). We and

others have shown the expression of pericyte markers in PDLSCs, but their functional roles *in vivo* have not yet been identified. The *in vivo* Matrigel plus assay is useful to confirm the *in vivo* angiogenic potential of PDLSCs as a perivascular cell source. In our study, the injection of PDLSCs alone or HUVECs alone did not result in the formation of vessel-like structures *in vivo*. This result is in accordance with previous reports that suggested the necessity of co-injection with endothelial cell sources (Kim et al., 2016; Melero-Martin et al., 2008). However, when PDLSCs and HUVECs were co-injected into immunodeficient mice, vessel-like structures were readily formed and host blood cells were observed in the lumen. Moreover, immunofluorescent data revealed that PDLSCs co-localized within the perivascular region near HUVECs. These data suggested that PDLSCs could have a functional role as perivascular cells for *in vivo* angiogenesis and reciprocal interactions between PDLSCs and HUVECs need to be identified.

The effects of SDF-1α-CXCR4 axis have been reported in dental pulp stem cells, which are involved in the proliferation, differentiation, and recruitment (Gong et al., 2010; Jiang et al., 2008; 2012). SDF-1α increased the proliferation of and stimulated the migration of PDLSCs (Du et al., 2012). In our results, PDLSCs showed contradictory expression pattern of SDF-1α and CXCR4 which is in accordance with a previous report (Trubiani et al., 2008). These reports suggest important roles for SDF-1α in PDLSCs. In contrast, HUVECs showed high expression of CXCR4 but low expression of SDF-1α. This suggested functional roles of the SDF-1α and CXCR4 axis between PDLSCs and HUVECs. Actually, treatment with AMD3100, a CXCR4 antagonist, reduced *in vivo* vessel formation in the Matrigel plug assay. These results confirmed the involvement of the SDF-1α and CXCR4 axis in *in vivo* vessel formation by PDLSCs and HUVECs.

Recently, the preclinical efficacy in other diseases, such as experimental autoimmune encephalomyelitis, was investigated using PDLSCs (Trubiani et al., 2016). Moreover, the immunomodulatory capacity of PDLSCs was also reported (Wada et al., 2009; 2013). Our work revealed that PDLSCs could be used as perivascular sources to form functional vessel-like structures *in vivo*. The SDF-1α and CXCR4 axis appears to be an important mediator of *in vivo* angiogenesis, although other angiogenic factors (chemokines and cytokines) from PDLSCs could also be helpful for *in vivo* angiogenesis with HUVECs. Considering the importance of vessel formation in tissue engineering, PDLSCs will be important candidates for not only tissue engineering requiring high vasculature but also disordered vessel diseases such as cerebrovascular diseases.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

ACKNOWLEDGEMENTS

This study was supported by National Research Foundation (NRF-2016R1A6A3A11930396 and NRF-2016R1A5A2945889) and National Research Foundation of Korea Grant, through the Bio and Medical Technology Development Program of the National Research Foundation funded by the Ministry of Science, ICT and Future Planning (2013M3A9B2076480).

REFERENCES

Aiuti, A., Webb, I.J., Bleul, C., Springer, T., and Gutierrez-Ramos, J.C. (1997). The chemokine SDF-1 is a chemoattractant for human CD34+ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34+ progenitors to peripheral blood. J. Exp. Med. *185*, 111-120.

Armulik, A., Abramsson, A., and Betsholtz, C. (2005). Endothelial/pericyte interactions. Circ. Res. *97*, 512-523.

Au, P., Daheron, L.M., Duda, D.G., Cohen, K.S., Tyrrell, J.A., Lanning, R.M., Fukumura, D., Scadden, D.T., and Jain, R.K. (2008). Differential *in vivo* potential of endothelial progenitor cells from human umbilical cord blood and adult peripheral blood to form functional long-lasting vessels. Blood *111*, 1302-1305.

Chen, F.M., and Jin, Y. (2010). Periodontal tissue engineering and regeneration: current approaches and expanding opportunities. Tissue Eng. Part B Rev. *16*, 219-255.

Corselli, M., Chen, C.W., Crisan, M., Lazzari, L., and Peault, B. (2010). Perivascular ancestors of adult multipotent stem cells. Arterioscler. Thromb. Vasc. Biol. *30*, 1104-1109.

Crisan, M., Yap, S., Casteilla, L., Chen, C.W., Corselli, M., Park, T.S., Andriolo, G., Sun, B., Zheng, B., Zhang, L., et al. (2008). A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell *3*, 301-313.

Du, L., Yang, P., and Ge, S. (2012). Stromal cell-derived factor-1 significantly induces proliferation, migration, and collagen type I expression in a human periodontal ligament stem cell subpopulation. J. Periodontol. *83*, 379-388.

Gaengel, K., Genove, G., Armulik, A., and Betsholtz, C. (2009). Endothelial-mural cell signaling in vascular development and angiogenesis. Arterioscler Thromb. Vasc. Biol. *29*, 630-638.

Gong, Q.M., Quan, J.J., Jiang, H.W., and Ling, J.Q. (2010). Regulation of the stromal cell-derived factor-1alpha-CXCR4 axis in human dental pulp cells. J. Endod. *36*, 1499-1503.

Huang, C., Gu, H., Yu, Q., Manukyan, M.C., Poynter, J.A., and Wang, M. (2011). Sca-1+ cardiac stem cells mediate acute cardioprotection via paracrine factor SDF-1 following myocardial ischemia/reperfusion. PLoS One *6*, e29246.

Isner, J.M., and Asahara, T. (1999). Angiogenesis and vasculogenesis as therapeutic strategies for postnatal neovascularization. J. Clin. Invest. *103*, 1231-1236.

Isner, J.M., Pieczek, A., Schainfeld, R., Blair, R., Haley, L., Asahara, T., Rosenfield, K., Razvi, S., Walsh, K., and Symes, J.F. (1996). Clinical evidence of angiogenesis after arterial gene transfer of phVEGF165 in patient with ischaemic limb. Lancet *348*, 370-374.

Ivanovski, S., Gronthos, S., Shi, S., and Bartold, P.M. (2006). Stem cells in the periodontal ligament. Oral. Dis. *12*, 358-363.

Iwasaki, K., Komaki, M., Yokoyama, N., Tanaka, Y., Taki, A., Kimura, Y., Takeda, M., Oda, S., Izumi, Y., and Morita, I. (2013). Periodontal ligament stem cells possess the characteristics of pericytes. J. Periodontol. *84*, 1425-1433.

Jaerve, A., Schira, J. and Muller, H.W. (2012). Concise review: the potential of stromal cell-derived factor 1 and its receptors to promote stem cell functions in spinal cord repair. Stem Cells Transl. Med. *1*, 732-739.

Jain, R.K., Au, P., Tam, J., Duda, D.G. and Fukumura, D. (2005). Engineering vascularized tissue. Nat. Biotechnol. *23*, 821-823.

Jiang, L., Zhu, Y.Q., Du, R., Gu, Y.X., Xia, L., Qin, F. and Ritchie, H.H. (2008). The expression and role of stromal cell-derived factor-1alpha-

CXCR4 axis in human dental pulp. J. Endod. *34*, 939-944.

Jiang, L., Peng, W.W., Li, L.F., Yang, Y. and Zhu, Y.Q. (2012). Proliferation and multilineage potential of CXCR4-positive human dental pulp cells *in vitro*. J. Endod. *38*, 642-647.

Kim, J.H., Kim, G.H., Kim, J.W., Pyeon, H.J., Lee, J.C., Lee, G. and Nam, H. (2016). *In vivo* angiogenic capacity of stem cells from human exfoliated deciduous teeth with human umbilical vein endothelial Cells. Mol. Cells *39*, 790-796.

Lee, J.Y., Nam, H., Park, Y.J., Lee, S.J., Chung, C.P., Han, S.B., and Lee, G. (2011). The effects of platelet-rich plasma derived from human umbilical cord blood on the osteogenic differentiation of human dental stem cells. In Vitro Cell Dev. Biol. Anim. *47*, 157-164.

Maeda, H., Tomokiyo, A., Fujii, S., Wada, N., and Akamine, A. (2011). Promise of periodontal ligament stem cells in regeneration of periodontium. Stem Cell Res. Ther. *2*, 33.

Melero-Martin, J.M., Khan, Z.A., Picard, A., Wu, X., Paruchuri, S. and Bischoff, J. (2007). *In vivo* vasculogenic potential of human blood-derived endothelial progenitor cells. Blood *109*, 4761-4768.

Melero-Martin, J.M., De Obaldia, M.E., Kang, S.Y., Khan, Z.A., Yuan, L., Oettgen, P., and Bischoff, J. (2008). Engineering robust and functional vascular networks *in vivo* with human adult and cord blood-derived progenitor cells. Circ. Res. *103*, 194-202.

Mohle, R., Bautz, F., Rafii, S., Moore, M.A., Brugger, W., and Kanz, L. (1998). The chemokine receptor CXCR-4 is expressed on CD34+ hematopoietic progenitors and leukemic cells and mediates transendothelial migration induced by stromal cell-derived factor-1. Blood *91*, 4523-4530.

Nagatomo, K., Komaki, M., Sekiya, I., Sakaguchi, Y., Noguchi, K., Oda, S., Muneta, T., and Ishikawa, I. (2006). Stem cell properties of human periodontal ligament cells. J. Periodontal. Res. *41*, 303-310.

Petit, I., Jin, D., and Rafii, S. (2007). The SDF-1-CXCR4 signaling pathway: a molecular hub modulating neo-angiogenesis. Trends Immunol. *28*, 299-307.

Ren, S., and Duffield, J.S. (2013). Pericytes in kidney fibrosis. Curr. Opin. Nephrol. Hypertension *22*, 471-480.

Seo, B.M., Miura, M., Gronthos, S., Bartold, P.M., Batouli, S., Brahim, J., Young, M., Robey, P.G., Wang, C.Y., and Shi, S. (2004). Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet *364*, 149-155.

Shi, S., and Gronthos, S. (2003). Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. J. Bone Miner. Res. *18*, 696-704.

Teicher, B.A., and Fricker, S.P. (2010). CXCL12 (SDF-1)./CXCR4 pathway in cancer. Clin. Cancer Res. *16*, 2927-2931.

Trubiani, O., Isgro, A., Zini, N., Antonucci, I., Aiuti, F., Di Primio, R., Nanci, A., Caputi, S., and Paganelli, R. (2008). Functional interleukin-7/interleukin-7Ralpha, and SDF-1alpha/CXCR4 are expressed by human periodontal ligament derived mesenchymal stem cells. J. Cell Physiol. *214*, 706-713.

Trubiani, O., Giacoppo, S., Ballerini, P., Diomede, F., Piattelli, A., Bramanti, P., and Mazzon, E. (2016). Alternative source of stem cells derived from human periodontal ligament: a new treatment for experimental autoimmune encephalomyelitis. Stem Cell Res Ther. *7*, 1.

Wada, N., Menicanin, D., Shi, S., Bartold, P.M., and Gronthos, S. (2009). Immunomodulatory properties of human periodontal ligament stem cells. J. Cell Physiol. *219*, 667-676.

Wada, N., Gronthos, S., and Bartold, P.M. (2013). Immunomodulatory effects of stem cells. Periodontol. *2000 63*, 198-216.

Wang, K., Zhao, X., Kuang, C., Qian, D., Wang, H., Jiang, H., Deng, M., and Huang, L. (2012). Overexpression of SDF-1alpha enhanced migration and engraftment of cardiac stem cells and reduced infarcted size via CXCR4/PI3K pathway. PLoS One *7*, e43922.

Zemani, F., Silvestre, J.S., Fauvel-Lafeve, F., Bruel, A., Vilar, J., Bieche, I., Laurendeau, I., Galy-Fauroux, I., Fischer, A.M., and Boisson-Vidal, C. (2008). *Ex vivo* priming of endothelial progenitor cells with SDF-1 before transplantation could increase their proangiogenic potential. Arterioscler Thromb. Vasc. Biol. *28*, 644-650.

Zhu, W., and Liang, M. (2015). Periodontal ligament stem cells: current status, concerns, and future prospects. Stem Cells Int. *2015*, 972313.