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Role of pigment epithelium-derived factor in the involution of hemangioma: Autocrine growth inhibition of hemangioma-derived endothelial cells

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ABSTRACT

Hemangioma is a benign tumor derived from abnormal blood vessel growth. Unlike other vascular tumor counterparts, a hemangioma is known to proliferate during its early stage but it is followed by a stage of involution where regression of the tumor occurs. The critical onset leading to the involution of hemangioma is currently not well understood. This study focused on the molecular identities of the involution of hemangioma. We demonstrated that a soluble factor released from the involuting phase of hemangioma-derived endothelial cells (HemECs) and identified pigment epithelium-derived factor (PEDF) as an anti-angiogenic factor that was associated with the growth inhibition of the involuting HemECs. The growth inhibition of the involuting HemECs was reversed by suppression of PEDF in the involuting HemECs. Furthermore, we found that PEDF was more up-regulated in the involuting phase of hemangioma tissues than in the proliferating or the involuted. Taken together, we propose that PEDF accelerates the involution of hemangioma by growth inhibition of HemECs in an autocrine manner. The regulatory mechanism of PEDF expression could be a potential therapeutic target to treat hemangiomas.

1. Introduction

Hemangioma is one of the most common tumors found in infancy, but its underlying cause remains unknown. Hemangioma is a type of benign vascular tumor resulting from abnormal growth of blood vessels where the endothelial cell lining of the vessels undergoes continuous angiogenesis and proliferation [1]. Infantile hemangioma (IH) tends to occur mainly on the skin usually around the face and neck area. Additionally, being a benign tumor, IH may be considered harmless; however, depending on the location of the hemangioma, vision- or life-threatening symptoms can occur [2]. To date, the treatment of IH includes surgical removal, corticosteroid treatments, and propranolol treatments as a first-line therapy for intervention. However, long-term treatment with corticosteroids may cause side effects such as immune suppression and delayed skeletal growth [3]. Moreover, as a relatively new pharmacologic treatment for IH, propranolol has an unclear mechanism of action and the potential to cause serious side effects such as bradycardia and hypotension [4]. Thus, novel therapeutic modalities to treat IH are needed.

Hemangioma is unique compared with cancer and other vascular tumor counterparts because of a distinct characteristic of an intrin-

Abbreviations: HemECs, hemangioma-derived endothelial cells; HUVECs, human umbilical vein endothelial cells; IH, infantile hemangioma; PEDF, pigment epithelium-derived factor; VEGF, vascular endothelial growth factor.

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sic mechanism of involution [5,6]. During the few months after birth, endothelial cells in hemangioma proliferate rapidly. Next, spontaneous involution for several years of childhood is followed where natural regression of the tumor occurs. This unique process of involution occurs spontaneously; the cause and onset is not well understood [7]. In the involuting phase, the population of endothelial cells begins to decrease. This is followed by the involuted phase, in which endothelial cells are almost not present and the tumor is replaced by fatty tissue with only a minor scar remaining [8].

Understanding this natural process of vascular regression in IH is important because it possesses the potential to treat hemangiomas through the strategy of accelerating the process of involution. Despite the importance of the induction of hemangioma involution, the mechanisms by which the involution is initiated remains largely unknown. Vascular endothelial growth factor (VEGF) [9] and platelet-derived growth factor [10] are potent stimulator of endothelial cell proliferation and the expression of those factors parallels the proliferating phase of IH [11,12]; thus, the inhibition of these factors may be effective to prevent the proliferation rather than induction of the involution of IH [12,13]. Although many pharmacologic approaches to treat IH target proliferating endothelial cells by inhibiting those growth factors, fewer approaches identify the novel regulators of the involution of IH. Given the spontaneous occurrence of the involuting phase [7] and the possibility of the onset of an endogenous mechanism in IH [14], we hypothesized that certain autocrine factors would be associated with the inhibition of proliferating endothelial cells during the involution phase of hemangioma.

In the present study, we investigated the negative regulators of hemangioma growth secreted from hemangioma-derived endothelial cells (HemECs). We found that pigment-epithelium derived factor (PEDF) inhibited the growth of the involuting HemECs in an autocrine manner and that PEDF expression was up-regulated during the involuting phase of IH tissues. To our knowledge, this is the first report highlighting the role of PEDF in the involution of IH as a therapeutic target.

2. Materials and methods

2.1. Cell culture

All of the experiments in this study were obtained from patients with informed written consent and protocols for the study approved by the Institutional Review Board of Seoul National University Hospital (IRB#H-1012-060-344). From three patients with proliferating, involuting, and involuted hemangiomas, we isolated hemangioma-derived endothelial cells. Briefly, the hemangioma tissues were extracted from the patients and washed. The blood vessel was isolated using a blade and incubated in 0.02% collagenase type II (Worthington, Lakewood, NJ, USA) for 30 min at 37 °C with gentle shaking. The digested tissues were further incised and washed in Dulbecco's Modified Eagle's Medium (Thermo Fisher Scientific, Waltham, MA, USA). After centrifugation at 2000 rpm for 3 min. The pellet was resuspended in Endothelial Growth Media-2 (EGM-2; Lonza, Walkersville, USA) and seeded in 60-mm dishes in preparation for cell sorting. HemECs were isolated by FACS using human CD31 monoclonal antibodies (BD Biosciences, San Jose, CA, USA) and CD31-negative non-HemEC cells were designated as HemEC(-).

Human umbilical vein endothelial cells (HUVECs) were purchased from Lonza and maintained in EGM-2 with 5% FBS. All of the cells were cultured at 37 °C in an incubator with a humidified atmosphere of 95% O_2 and 5% CO_2.

2.2. Cell counting

Cells (3×10^4 cells) were seeded on separate gelatin (Welgene, Korea)-coated 12-well plates (SPL, Korea). All of the plates were incubated with EGM-2 for 72 h, while one set of plates had its media changed every 24 h. Cells were trypsinized (Gibco, Grand Island, NY, USA) and counted every 24 h using a hemocytometer (Marienfeld Superior, Lauda-Königshofen, Germany) and Trypan blue (Welgene).

2.3. MTT assay

Conditioned media were collected from HemECs after 24 h and 48 h of incubation. The conditioned media were mixed with fresh EGM-2 media at a ratio of 1:1 and added to HUVECs. After 48 h of incubation, MTT (Sigma–Aldrich, St. Louis, MO, USA) was added to the media, and the mixture was incubated for 4 h. The resulting precipitates were dissolved in isopropanol, and formazan levels were determined at 570 nm.

2.4. Polymerase chain reaction (PCR)

Total RNA was extracted from the cells using the RNeasy kit (Qiagen, CA, USA) according to the manufacturer's instructions. The cDNA was synthesized using an oligo(dT) primer and Superscript reverse transcriptase (Invitrogen). The cDNA was then used to amplify genes of interest using GoTaq Polymerase (Promega) and a MyCycler™ system (BioRad). The PCR products were analyzed using 2% agarose gel electrophoresis and SYBR Safe (BioRad). The gel was then filmed using ChemiDoc XPS+ (BioRad).

For real-time quantitative PCR, the SYBR Green Super Mix (BioRad) and an i-Cycler PCR thermocycler (BioRad) were used. Samples containing 20 ng of cDNA were analyzed in triplicate. The values were normalized to those of β-actin expression levels. The sequences of PCR primers are summarized in Table 1.

Table 1
Nucleotide sequences of primers used in experiments.

Targets of PCR	Forward primers (5′–3′)	Reverse primers (5′–3′)
PEDF	TTCCTGCCCCTGAAAGTGAC	GGACTTGGTGACTTCGCCTT
CD31	GCTCTCTTGATCATTGCG	GAGGACACTTGAACTTCC
VE-cadherin	GGCAAGATCAAGTCAAGCGTG	ACGTCTCCTGTCTCTGCATCG
VEGF	GCAGAAGGAGGAGGGCAGAAT	GCACACAGGATGGCTTGAAGA
E-selectin	CCGAGCGAGGCTACATGAAT	GCCACATTGGAGCCTTTTGG
GLUT1	CTTTTCTGTTGGGGGCATGAT	CCGCAGTACACACCGATGAT
PCNA	AGGGCTCCATCCTCAAGAAGG	TGGTGCTTCAAATACTAGCGC
β-actin	TCCCTGGAGAAGAGCTACGA	AGCACTGTGTTGGCGTACAG
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC

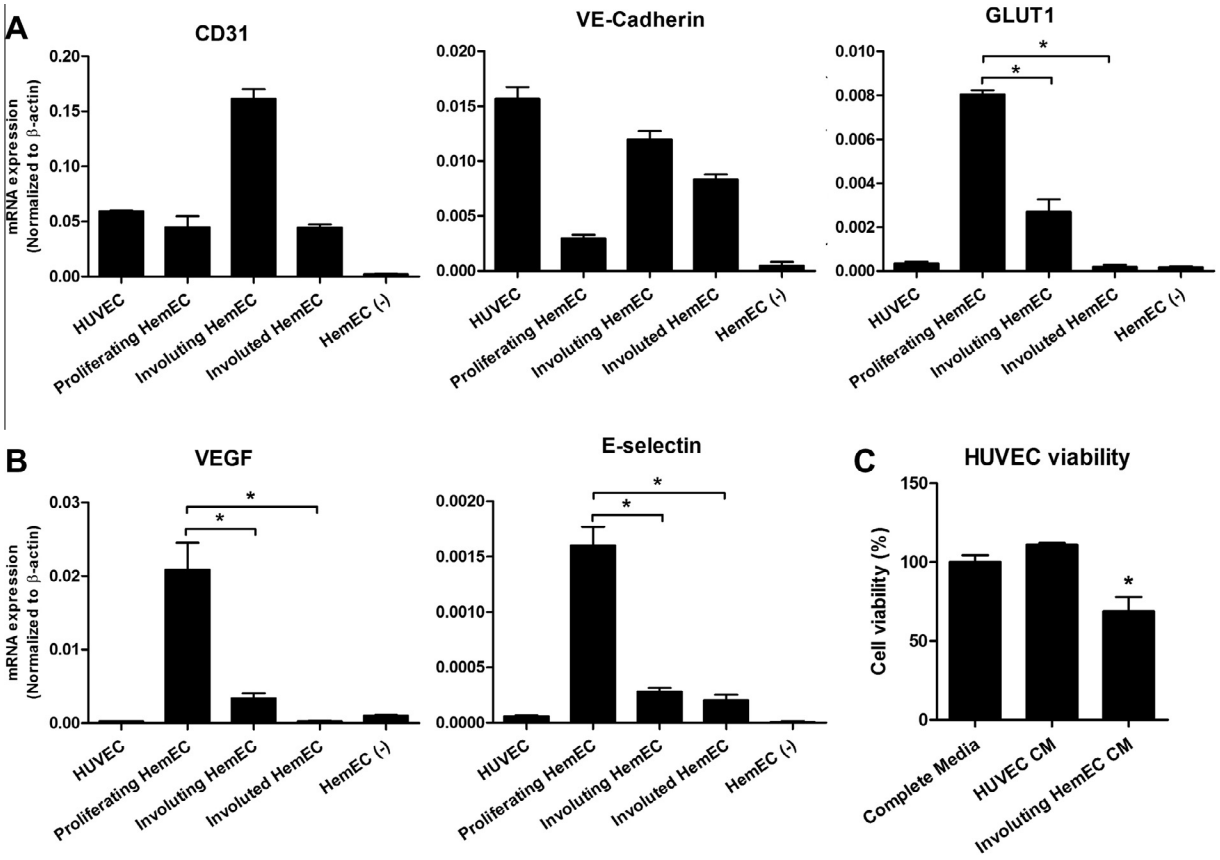

Fig. 1. Isolation of the involuting HemECs and the effect of the culture conditioned media on cell proliferation. The mRNA transcript levels of endothelial specific markers (CD31, VE-cadherin, and GLUT1) (A) or phase-specific markers (VEGF and E-selectin) (B) were measured by quantitative RT-PCR. The mRNA expression levels were normalized to those of the β-actin control. *denotes $P < 0.05$ between the two values ($n = 3$). (C) The culture conditioned media from the involuting HemECs and HUVECs cultured for 48 h were treated to HUVECs for 24 h. The complete media without incubation was used as control. Cell viability was then assessed by MTT staining. Results are given as percentages versus the control complete media value and plotted as the mean ± SD. *denotes $P < 0.05$ versus the value of control complete media ($n = 5$).

2.5. Human angiogenesis cytokine array

The expression of cytokines in the cell culture supernatants was assessed using the Human Angiogenesis Cytokine Array kit (BD Biosciences). Conditioned media without serum were collected from HUVECs and HemECs after 48 h of incubation. The membrane blots of the conditioned media were developed according to the manufacturer's instructions and were analyzed by measuring the density of each spot compared with the negative and positive controls.

2.6. siRNA

PEDF siRNAs (siPEDF#1 and siPEDF#3) and negative-control siRNA (siControl) (IDT™ Inc., Coralville, IA, USA) were dissolved in Opti-Mem (Gibco) and Lipofectamine 2000 (Invitrogen). The mixture was added to HemECs and incubated at 37 °C overnight. The total RNA was extracted from the cells to confirm PEDF knockdown in cells by RT-PCR. The transfected cells were then used for cell counting, the MTT assay and immunofluorescence.

2.7. Immunohistochemistry

Tissue sections from the patients of each phase of IH were deparaffinized and processed for immunohistochemical staining. The sections were blocked with 5% goat serum and then were incubated with the primary antibody against human PEDF (R&D Systems). The sections were incubated with anti-mouse IgG antibody and detected using DAB (Vector Laboratories, Burlingame, CA, USA) with hematoxylin (Sigma–Aldrich) counterstaining. Images were obtained using an Olympus BX51 microscope equipped with OlyVia software (Olympus, Germany).

2.8. Statistical analysis

The data are presented as the mean ± SD. The statistical analyses were performed using Prism software 5.0 (GraphPad Software, Inc., San Diego, CA, USA), and Student's *t*-test was used to compare the differences between groups; a *P* value less than 0.05 was considered statistically significant.

3. Results

3.1. Isolated HemECs express autocrine factors to inhibit their own cell proliferation

To verify the phase-specific HemECs, we analyzed the expression of the endothelial-specific and phase-specific markers by quantitative RT-PCR. All HemECs expressed CD31 and VE-cadherin transcripts, whereas HemEC-negative cells did not (Fig. 1A). Because GLUT1 expression distinguishes hemangioma from other vascular anomalies [15], we examined and confirmed that the proliferating and involuting HemECs expressed GLUT1 significantly higher than HUVECs or HemEC-negative cells (Fig. 1A). To determine whether HemECs exhibit phase-specific markers, we

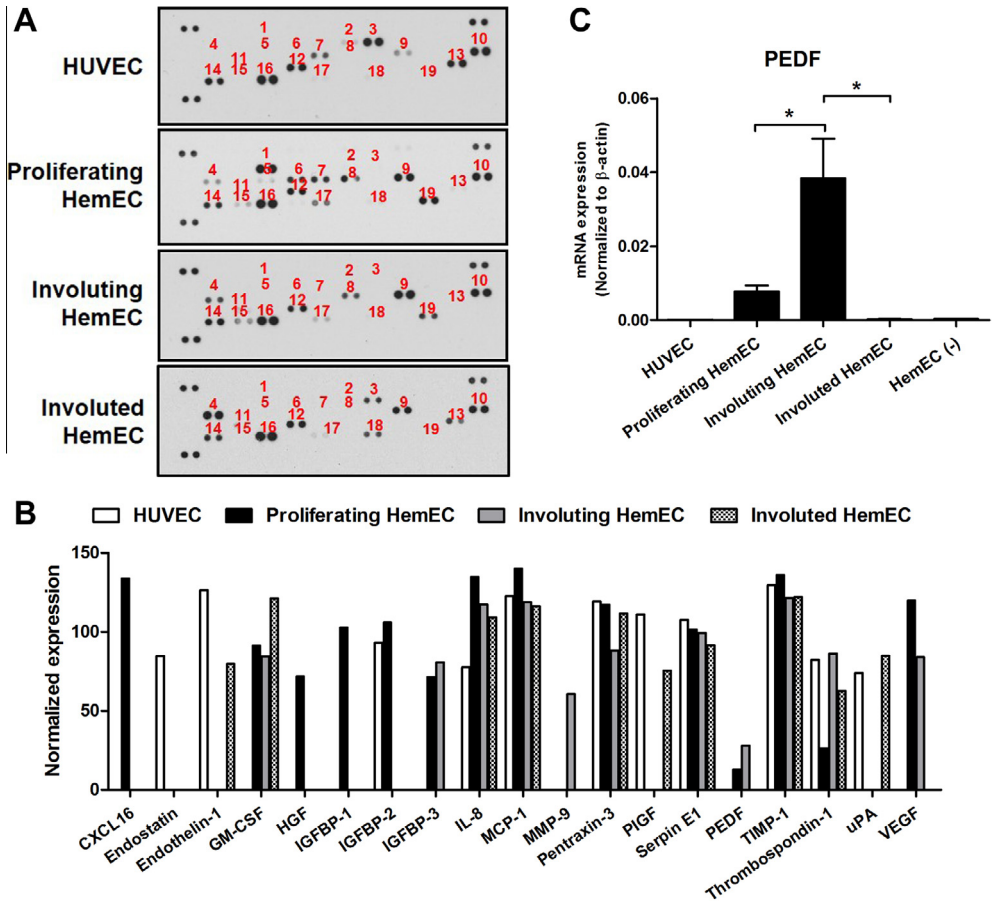

Fig. 2. Identification of autocrine factors in HemECs and their culture supernatants. (A) Human Angiogenesis Cytokine Array evaluating pro- and anti-angiogenic protein expression changes in the culture conditioned media from the proliferating, involuting and involuted HemECs, and HUVECs after 48 h of incubation. (B) Quantification of the protein expression in (A). Results are given as the mean dot intensity and are expressed as a percentage of the standard intensities. (C) PEDF gene expression was measured by quantitative RT-PCR. *denotes $P < 0.05$ between the two values ($n = 3$).

analyzed the gene expression of VEGF and E-selectin (Fig. 1B). Consistent with previous reports [11,13,16,17], the proliferating HemECs expressed more VEGF and E-selectin transcripts than the involuting and involuted HemECs (Fig. 1B), providing further confirmation of a phase-specific phenotype of HemECs.

To examine whether the involuting HemECs release certain factors that affect the cell growth, we treated the culture conditioned media from the involuting HemECs to normal endothelial cells (HUVECs) and measured the cell viability of HUVECs. We observed that the conditioned media incubated with the involuting HemECs for 48 h significantly inhibited the HUVEC viability, while the conditioned media with HUVECs did not (Fig. 1C). These results demonstrate that the involuting HemECs release certain factors that could inhibit the cell growth themselves.

3.2. Pigment-epithelium derived factor is identified as an anti-angiogenic molecule from the involuting HemECs

To identify the autocrine factors that induce growth inhibition of the involuting HemECs, we examined the expression profiles of angiogenesis-related proteins in the conditioned media from different phases of HemECs using human angiogenesis cytokine array. Among 55 angiogenesis-related proteins blotted in the array, 19 proteins were detected in the conditioned media (Fig. 2A). We found that anti-angiogenic factors including tissue inhibitor of metalloproteinase-1 (TIMP-1) and thrombospondin-1 were expressed in all HemECs and HUVECs, while PEDF was mainly expressed in the involuting HemECs (Fig. 2A and B). The predominant expression of PEDF in the involuting HemECs was also confirmed by quantitative RT-PCR analysis (Fig. 2C). We also confirmed the increased expression of VEGF protein in the proliferating and involuting HemECs compared to that in the involuted HemECs and HUVECs (Fig. 2A and B), as was observed in RT-PCR analysis (Fig. 1B). Based on the findings that PEDF is anti-angiogenic [18], and its expression is highly induced in the involuting HemECs (Fig. 2), we hypothesized that PEDF could be induced during and could play an important role in the involution of IH.

3.3. PEDF is anti-angiogenic and is associated with the growth inhibition of HemECs

To determine whether PEDF plays an inhibitory role in endothelial cell growth, we first treated PEDF to normal endothelial cells (HUVECs), which does not express PEDF protein (Fig. 2). Exogenous PEDF treatment decreased HUVEC viability in dose and time dependent manners (Fig. 3A and B), indicating that PEDF can suppress normal endothelial cell growth as an anti-angiogenic factor.

We further asked whether endogenous PEDF from the involuting HemECs is associated with the growth inhibition of the involuting HemECs. We treated the involuting HemECs with either control siRNA (siControl) or two different PEDF siRNAs (siPEDF#1 and siPEDF#3). Both PEDF targeting siRNA efficiently reduced PEDF expression; but siPEDF#3 more efficiently suppressed PEDF expression (Fig. 3C and D). Thus, siPEDF#3 was used in other experiments. When the PEDF expression was suppressed in the

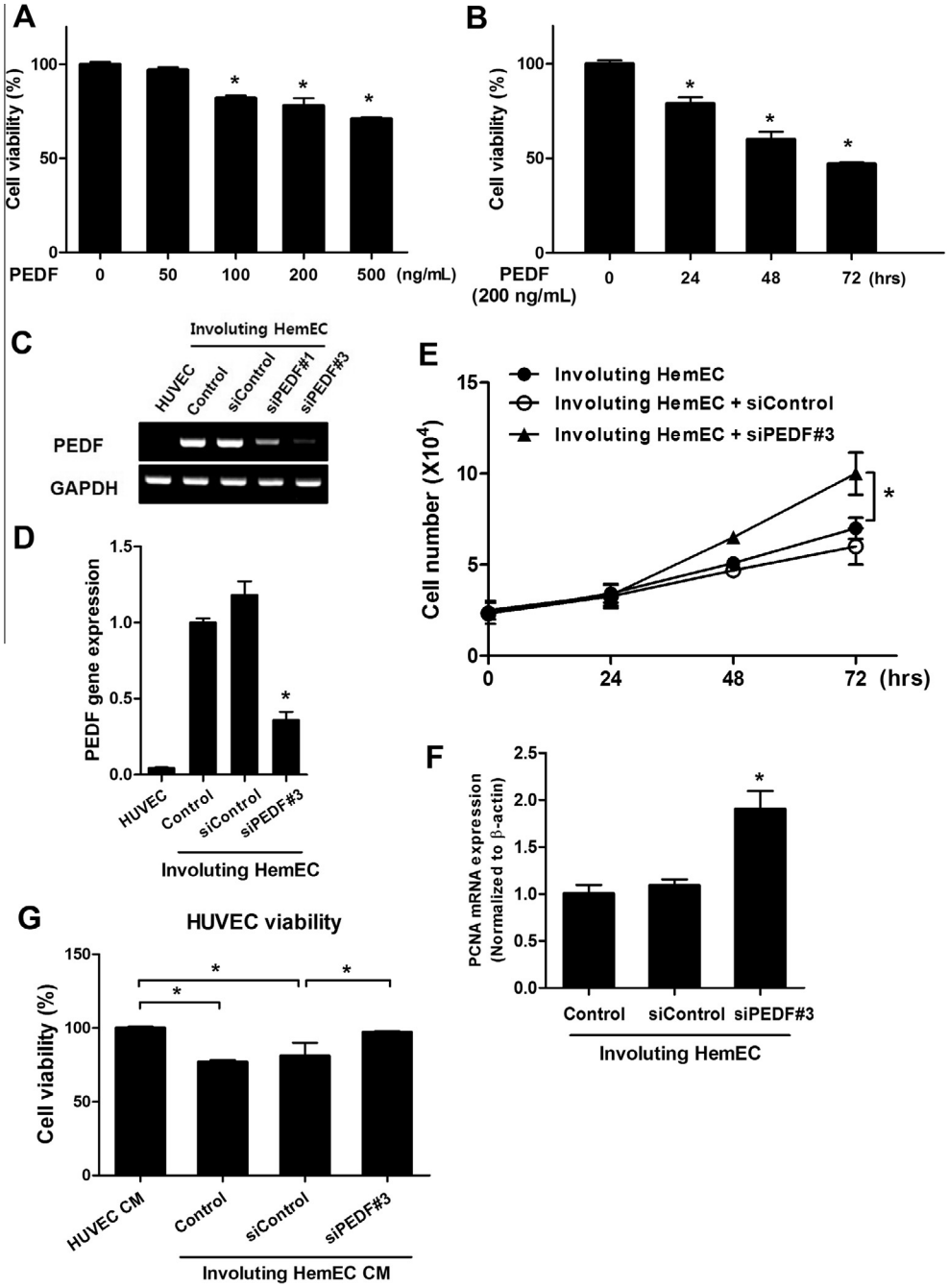

Fig. 3. Effect of PEDF on endothelial cell viability and HemEC proliferation. (A) Dose- and (B) time-dependent effects of PEDF on HUVEC viability. HUVEC viability was assessed by MTT analysis. Results are plotted as the mean ± SD. *denotes $P < 0.05$ versus the value of 0 concentration or time ($n = 5$). (C, D) Transfection of siRNA for PEDF reduced PEDF gene expression. Conventional (C) and quantitative (D) RT-PCR showed the effective down-regulation of PEDF gene expression by siPEDF#3. *denotes $P < 0.05$ versus the value of the involuting HemECs transfected with control siRNA (siControl) ($n = 5$). (E) After the transfection of siControl and siPEDF#3 in the involuting HemECs, the cells were incubated for the indicated times. Cell proliferation was assessed by counting cell numbers. *denotes $P < 0.05$ between the two values ($n = 5$). (F) Quantitative RT-PCR analysis of PCNA gene expression in the involuting HemECs transfected with siControl and siPEDF#3. *denotes $P < 0.05$ versus the value of siControl ($n = 5$). (G) The culture conditioned media from the involuting HemECs transfected with either siControl or siPEDF#3 were incubated with HUVECs for 24 h. HUVEC viability was assessed by MTT analysis. Results are plotted as the mean ± SD. *denotes $P < 0.05$ between the two values ($n = 5$).

involuting HemECs, the cell proliferation of the involuting HemECs was significantly increased (Fig. 3E). The increased cell proliferation in siPEDF#3-treated involuting HemECs was also confirmed by the increased expression of PCNA mRNA—a marker of cell proliferation (Fig. 3F). These results suggest that PEDF secreted from the involuting HemECs limits their own cell proliferation.

Next, to determine whether endogenous PEDF from the involuting HemECs mediates the growth inhibition of HemECs rather than affects the intracellular machinery for cell growth, we treated the conditioned media from the siPEDF-treated involuting HemECs to normal endothelial cells (HUVECs). The decreased HUVEC viability induced by the conditioned media from the involuting HemECs was abolished by that from siPEDF-treated HemECs (Fig. 3G). Taken together, these results suggest that the involuting HemECs release PEDF as a growth inhibitory factor to account for the impaired cell proliferation of the involuting HemECs.

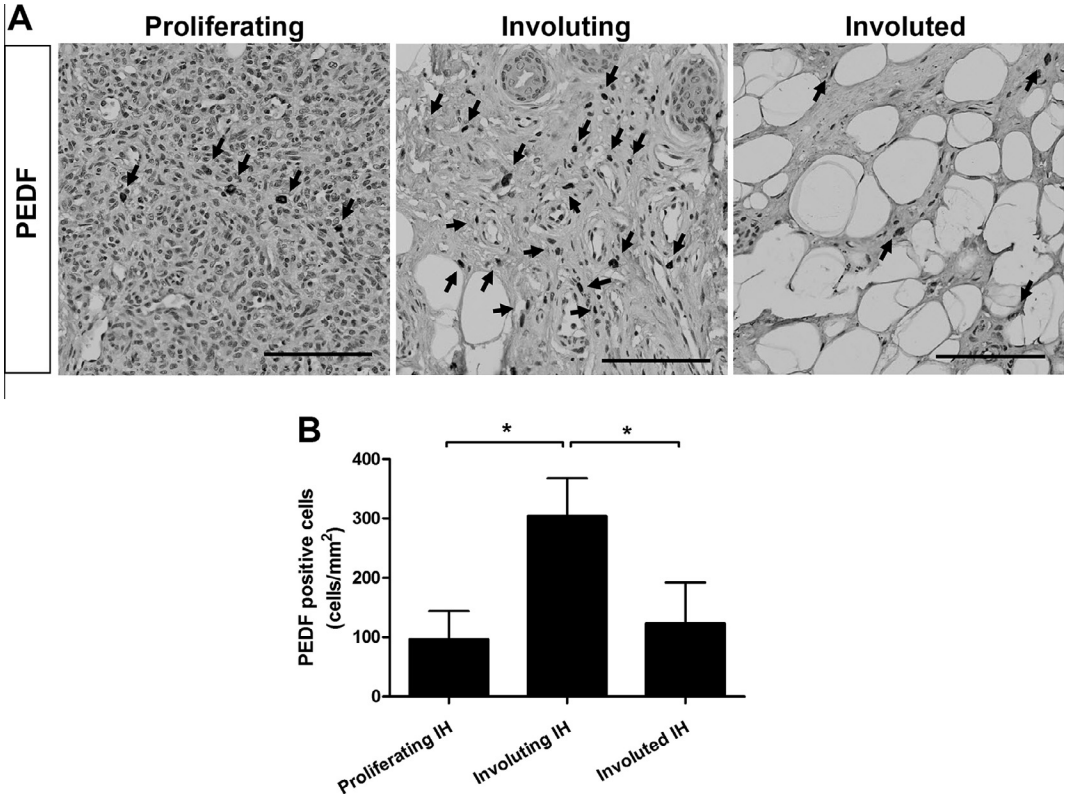

Fig. 4. Expression of PEDF in hemangioma tissue sections. (A) Representative immunohistochemical staining of PEDF in the proliferating, involuting and involuted phase of IH tissue sections. The arrows indicate PEDF-positive cells. Bar = 100 μm. (B) Quantification of PEDF-positive cells in each phase of the IH tissue sections. Results are plotted as the mean ± SD. *denotes $P < 0.05$ between the two values ($n = 3$).

3.4. Increased expression of PEDF coincides with the involuting phase of IH

Based on *in vitro* experiments, we next examined whether PEDF is increased in the involuting phase of IH *in vivo*. From three patient tissues per each phase of IH, we determined PEDF expression by immunochemical staining. PEDF expression was observed in all phases of IH; however, PEDF-positive cells were significantly higher in the involuting IH than in the proliferating and involuted IH phases (Fig. 4). This result suggests that PEDF is up-regulated during the involuting phase of IH tissue and could contribute to the growth inhibition of the involuting IH.

4. Discussion

In the present study, we screened the expression of anti-angiogenic factors to identify proteins that may be important for the involution of IH. We found that PEDF, an inhibitor of angiogenesis [18], was highly expressed in the involuting phase of HemECs and IH tissues and contributed to the growth inhibition of the involuting HemECs.

Pharmacological approaches to treat IH have focused more on the proliferating phases because angiogenic growth factors such as VEGF and FGF were expressed in the proliferating phases and subsequently diminished during the involution phases [11]. Recent studies have shown that VEGF is a major target of corticosteroid treatment by the inhibition of both the proliferation of HemECs and vasculogenic activity of hemangioma-derived stem cells [13,19,20]. However, given that 30% of IHs do not respond to corticosteroid treatment [16] and other therapies may be necessary, the induction of an intrinsic pathway of endothelial apoptosis could provide a novel therapeutic strategy for IH treatment. Thus, we focused on the proteins with an anti-angiogenic potential induced during the involuting phase that could cause the acceleration of the involution of IH.

Many approaches have revealed the cellular markers of the proliferating phase of IH: for example, VEGF [11,13] and E-selectin [21,22] are highly expressed in the proliferating phase but are down-regulated in the involuting phase. Consistently, we observed the increased expression of VEGF and E-selectin in the proliferating rather than the involuting HemECs (Fig. 1B). However, less is known concerning the cellular markers of the involuting phase of IH. We demonstrated here that PEDF is predominantly in the involuting HemECs (Fig. 2).

PEDF is a member of the serine protease inhibitor superfamily without an inhibitory function toward serine protease [23]. It was first discovered in human retinal cells [24,25] and was later found to have potent anti-angiogenic activity that prevented angiogenesis in the eye and tumors [18,26,27]. A growing body of evidence has shown the involvement of PEDF in the regulation of vascular integrity and angiogenesis. For example, PEDF promotes endothelial cell apoptosis through p38 MAPK signaling [28] and/or PPARδ-induced p53 activation [29]. PEDF also performs an anti-angiogenic function in VEGF- or bFGF-induced endothelial cell migration [18]. We also found that PEDF exerted anti-proliferating activity in HemECs as well as HUVECs (Fig. 3). Based on the previous reports and our findings, we assume that PEDF could contribute to the involution of HemEC growth. Although the induction mechanism of PEDF expression during the involuting phase remains to be addressed, these findings highlight a novel mechanism of PEDF to accelerate the involution of IH.

Interestingly, PEDF is expressed as an adipokine in adipocytes differentiated from mesenchymal stem cells [30,31]. Because the

involution of IH accompanies the appearance of adipocytes and the completely involuted hemangioma tissues are filled with fibrofatty tissues, the regulatory mechanism of the onset of IH involution may coincide with the onset of adipogenesis. Actually, the inhibition of β-adrenergic signaling with propranolol is effective in IH treatment by inducing both the apoptosis of HemECs and differentiation of hemangioma-derived stem cells into adipocytes [32]. These reports and our findings that PEDF was induced during the involuting phase of IH and inhibited the proliferation of HemECs suggest the possible involvement of PEDF not only in restraining the HemEC growth but also in initiating the adipogenic commitment.

In conclusion, we found that PEDF was highly expressed in the involuting phase of IH and plays a role in the involution of hemangioma. PEDF, as an anti-angiogenic factor, can decrease the proliferation of HemECs, a representative phenomenon during the process of involution of IH. We suggest that the regulatory mechanism of PEDF expression could be a potential therapeutic target to accelerate the regression of IH.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

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References

[1] T.L. Phung, M. Hochman, Pathogenesis of infantile hemangioma, Facial Plast. Surg. 28 (2012) 554–562.
[2] J.A. Bartlett, K.H. Riding, L.J. Salkeld, Management of hemangiomas of the head and neck in children, J. Otolaryngol. 17 (1988) 111–120.
[3] M.E. George, V. Sharma, J. Jacobson, S. Simon, A.J. Nopper, Adverse effects of systemic glucocorticosteroid therapy in infants with hemangiomas, Arch. Dermatol. 140 (2004) 963–969.
[4] M. de Graaf, J.M. Breur, M.F. Raphael, M. Vos, C.C. Breugem, S.G. Pasmans, Adverse effects of propranolol when used in the treatment of hemangiomas: a case series of 28 infants, J. Am. Acad. Dermatol. 65 (2011) 320–327.
[5] M.R. Ritter, R.A. Butschek, M. Friedlander, S.F. Friedlander, Pathogenesis of infantile hemangioma: new molecular and cellular insights, Expert Rev. Mol. Med. 9 (2007) 1–19.
[6] M.J. Razon, B.M. Kraling, J.B. Mulliken, J. Bischoff, Increased apoptosis coincides with onset of involution in infantile hemangioma, Microcirculation 5 (1998) 189–195.
[7] J.B. Mulliken, J. Glowacki, Hemangiomas and vascular malformations in infants and children: a classification based on endothelial characteristics, Plast. Reconstr. Surg. 69 (1982) 412–422.
[8] A. Kleiman, E.C. Keats, N.G. Chan, Z.A. Khan, Evolution of hemangioma endothelium, Exp. Mol. Pathol. 93 (2012) 264–272.
[9] N. Ferrara, H.P. Gerber, J. LeCouter, The biology of VEGF and its receptors, Nat. Med. 9 (2003) 669–676.
[10] E.J. Battegay, J. Rupp, L. Iruela-Arispe, E.H. Sage, M. Pech, PDGF-BB modulates endothelial proliferation and angiogenesis in vitro via PDGF beta-receptors, J. Cell Biol. 125 (1994) 917–928.
[11] K. Takahashi, J.B. Mulliken, H.P. Kozakewich, R.A. Rogers, J. Folkman, R.A. Ezekowitz, Cellular markers that distinguish the phases of hemangioma during infancy and childhood, J. Clin. Invest. 93 (1994) 2357–2364.
[12] E.E. Roach, R. Chakrabarti, N.I. Park, E.C. Keats, J. Yip, N.G. Chan, Z.A. Khan, Intrinsic regulation of hemangioma involution by platelet-derived growth factor, Cell Death Dis. 3 (2012) e328.
[13] S. Greenberger, E. Boscolo, I. Adini, J.B. Mulliken, J. Bischoff, Corticosteroid suppression of VEGF-A in infantile hemangioma-derived stem cells, N. Engl. J. Med. 362 (2010) 1005–1013.
[14] M.R. Ritter, M.I. Dorrell, J. Edmonds, S.F. Friedlander, M. Friedlander, Insulin-like growth factor 2 and potential regulators of hemangioma growth and involution identified by large-scale expression analysis, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 7455–7460.
[15] P.E. North, M. Waner, A. Mizeracki, M.C. Mihm Jr., GLUT1: a newly discovered immunohistochemical marker for juvenile hemangiomas, Hum. Pathol. 31 (2000) 11–22.
[16] A. Zvulunov, A. Metzker, Hemangiomas and vascular malformations: unapproved treatments, Clin. Dermatol. 20 (2002) 660–667.
[17] L.P. Abramson, M. Browne, V. Stellmach, J. Doll, M. Cornwell, M. Reynolds, R.M. Arensman, S.E. Crawford, Pigment epithelium-derived factor targets endothelial and epithelial cells in Wilms' tumor, J. Pediatr. Surg. 41 (2006) 1351–1356.
[18] D.W. Dawson, O.V. Volpert, P. Gillis, S.E. Crawford, H. Xu, W. Benedict, N.P. Bouck, Pigment epithelium-derived factor: a potent inhibitor of angiogenesis, Science 285 (1999) 245–248.
[19] M. Jinnin, D. Medici, L. Park, N. Limaye, Y. Liu, E. Boscolo, J. Bischoff, M. Vikkula, E. Boye, B.R. Olsen, Suppressed NFAT-dependent VEGFR1 expression and constitutive VEGFR2 signaling in infantile hemangioma, Nat. Med. 14 (2008) 1236–1246.
[20] L. Claesson-Welsh, Healing hemangiomas, Nat. Med. 14 (2008) 1147–1148.
[21] B.M. Kraling, M.J. Razon, L.M. Boon, D. Zurakowski, C. Seachord, R.P. Darveau, J.B. Mulliken, C.L. Corless, J. Bischoff, E-selectin is present in proliferating endothelial cells in human hemangiomas, Am. J. Pathol. 148 (1996) 1181–1191.
[22] D.M. Smadja, J.B. Mulliken, J. Bischoff, E-selectin mediates stem cell adhesion and formation of blood vessels in a murine model of infantile hemangioma, Am. J. Pathol. 181 (2012) 2239–2247.
[23] S.P. Becerra, A. Sagasti, P. Spinella, V. Notario, Pigment epithelium-derived factor behaves like a noninhibitory serpin. Neurotrophic activity does not require the serpin reactive loop, J. Biol. Chem. 270 (1995) 25992–25999.
[24] J. Tombran-Tink, L.V. Johnson, Neuronal differentiation of retinoblastoma cells induced by medium conditioned by human RPE cells, Invest. Ophthalmol. Vis. Sci. 30 (1989) 1700–1707.
[25] J. Tombran-Tink, G.G. Chader, L.V. Johnson, PEDF: a pigment epithelium-derived factor with potent neuronal differentiative activity, Exp. Eye Res. 53 (1991) 411–414.
[26] K. Mori, P. Gehlbach, S. Yamamoto, E. Duh, D.J. Zack, Q. Li, K.I. Berns, B.J. Raisler, W.W. Hauswirth, P.A. Campochiaro, AAV-mediated gene transfer of pigment epithelium-derived factor inhibits choroidal neovascularization, Invest. Ophthalmol. Vis. Sci. 43 (2002) 1994–2000.
[27] E. Alberdi, M.S. Aymerich, S.P. Becerra, Binding of pigment epithelium-derived factor (PEDF) to retinoblastoma cells and cerebellar granule neurons. Evidence for a PEDF receptor, J. Biol. Chem. 274 (1999) 31605–31612.
[28] L. Chen, S.S. Zhang, C.J. Barnstable, J. Tombran-Tink, PEDF induces apoptosis in human endothelial cells by activating p38 MAP kinase dependent cleavage of multiple caspases, Biochem. Biophys. Res. Commun. 348 (2006) 1288–1295.
[29] T.C. Ho, S.L. Chen, Y.C. Yang, C.L. Liao, H.C. Cheng, Y.P. Tsao, PEDF induces p53-mediated apoptosis through PPAR gamma signaling in human umbilical vein endothelial cells, Cardiovasc. Res. 76 (2007) 213–223.
[30] C. Chiellini, O. Cochet, L. Negroni, M. Samson, M. Poggi, G. Ailhaud, M.C. Alessi, C. Dani, E.Z. Amri, Characterization of human mesenchymal stem cell secretome at early steps of adipocyte and osteoblast differentiation, BMC Mol. Biol. 9 (2008) 26.
[31] S. Famulla, D. Lamers, S. Hartwig, W. Passlack, A. Horrighs, A. Cramer, S. Lehr, H. Sell, J. Eckel, Pigment epithelium-derived factor (PEDF) is one of the most abundant proteins secreted by human adipocytes and induces insulin resistance and inflammatory signaling in muscle and fat cells, Int. J. Obes. (Lond.) 35 (2011) 762–772.
[32] A. Wong, K.L. Hardy, A.M. Kitajewski, C.J. Shawber, J.K. Kitajewski, J.K. Wu, Propranolol accelerates adipogenesis in hemangioma stem cells and causes apoptosis of hemangioma endothelial cells, Plast. Reconstr. Surg. 130 (2012) 1012–1021.