Biochemical and Biophysical Research Communications 404 (2011) 103–108

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Clec14a is specifically expressed in endothelial cells and mediates cell to cell adhesion

Seung-Sik Rho [a], Hyun-Jung Choi [a], Jeong-Ki Min [b], Heon-Woo Lee [a], Hongryeol Park [a], Hyojin Park [a], Young-Myeong Kim [c], Young-Guen Kwon [a,*]

[a] Department of Biochemistry, College of Life Science and Biotechnology, Yonsei University, Seoul, Republic of Korea
[b] Therapeutic Antibody Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea
[c] Department of Molecular and Cellular Biochemistry, Kangwon National University, Chunchon, Republic of Korea

ARTICLE INFO

Article history:
Received 9 November 2010
Available online 21 November 2010

Keywords:
Clec14a
C-type lectin like domain
Endothelial cell
Cell–cell adhesion
Angiogenesis

ABSTRACT

Clec14a is a member of the thrombomodulin (TM) family, but its function has not yet been determined. Here, we report that Clec14a is a plasma membrane protein of endothelial cells (ECs) expressed specifically in the vasculature of mice. Deletion mutant analysis revealed that Clec14a mediates cell–cell adhesion through its C-type lectin-like domain. Knockdown of Clec14a in ECs suppressed cell migratory activity and filopodial protrusion, and delayed formation of tube-like structures. These findings demonstrate that Clec14a is a novel EC-specific protein that appears to play a role in cell–cell adhesion and angiogenesis.

1. Introduction

Clec14a belongs to a newly described C-type lectin domain superfamily that includes thrombomodulin (TM)/CD141, endosialin/TEM1/CD248, and CD93/C1qRP/AA4 [1]. These type I transmembrane proteins are classified based on their ectodomain structures consisting of a C-type lectin domain, a series of EGF domains, and a sushi-like domain [2]. The C-type lectin is a carbohydrate-binding protein domain. Proteins containing C-type lectin domains mediate a diverse range of functions including cell–cell adhesion, immune response to pathogens, and apoptosis [3,4]. For example, TM, which was originally identified in the vascular endothelium, is an anticoagulant factor. TM-deficient mice show spontaneous and fatal thrombosis in the arterial and venous circulation, due to unregulated activation of the coagulation system [5]. The C-type lectin domain of TM interferes with polymorphonuclear leukocyte adhesion to endothelial cells (ECs) [6] and counteracts the lipopolysaccharide-induced inflammatory response [7]. Endosialin was discovered as a human embryonic fibroblast-specific antigen and initially described as a marker of tumor endothelium [8,9]; it is not expressed on normal ECs or human umbilical vein endothelial cells (HUVECs) [10]. Because lack of endosialin can disrupt tumor growth and vascular differentiation, endosialin has generated interest as a target for antiangiogenic therapy [11]. Human CD93, a receptor involved in C1q-mediated enhancement of phagocytosis [12], is expressed on myeloid lineage cells, ECs, platelets, and microglia; however, CD93-deficient mice are viable and show no gross abnormalities in their vascular development. Taken together, these observations suggest a role for the TM family in the vasculature. The specific physiological role of Clec14a, however, remains unknown.

In the present study, we demonstrated that Clec14a is specifically expressed in the developing vasculature of mice and in human ECs. Results of an aggregation assay showed that Clec14a is involved in cell–cell adhesion via C-type lectin domain-mediated interaction. Furthermore, silencing of Clec14a *in vitro* attenuated EC angiogenic activity, as shown by cell migration and tube formation on Matrigel. These results suggest that Clec14a may play a significant role in endothelial cell–cell adhesion.

2. Materials and methods

2.1. Isolation and culture of ECs

HUVECs were isolated from human umbilical cord veins by collagenase, as described previously [13], and cells from passage 2 to passage 7 were used. HUVECs were cultured on plates 2% gelatin coated dishes at 37 °C in a 5% CO_2 humidified atmosphere using

Abbreviation: Clec14a, C-type lectin domain family 14, member a.

* Corresponding author. Address: Department of Biochemistry, College of Life Science and Biotechnology, Yonsei University, Seoul 120-749, Republic of Korea. Fax: +82 2 362 9897.
E-mail address: ygkwon@yonsei.ac.kr (Y.-G. Kwon).

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.11.075

M199 medium (Invitrogen, Carlsbad, CA) containing 20% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, 3 ng/mL basic fibroblast growth factor (R&D Systems, Minneapolis, MN), and 5 U/mL heprin.

2.2. Whole-mount in situ hybridization

Pregnant mouse (C57BL6) was sacrificed to prep embryos at embryonic day 10.5 (E 10.5). Whole-mount RNA *in situ* hybridization was performed essentially as described previously [14,15], using BM purple (Roche, Indianapolis, IN) as the color substrate. Mouse embryos were stored in 70% glycerol and photographed. The cDNA sequence of mouse Clec14a was used to transcribe cRNAs for *in situ* hybridization probes.

2.3. Immunostaining and fluorescence imaging

HUVECs grown on a 2% gelatin-coated glass-based dish were transfected with plasmids expressing Clec14a-GFP, Clec14aΔ^{cyto}-GFP, Clec14aΔ^{lectin}-GFP, or Clec14aΔ^{egf}-GFP as indicated in Fig. 2B. HUVECs were starved for 3 h in Medium 199 containing 1% FBS, then fixed in PBS containing 4% paraformaldehyde for 30 min at 4 °C, washed with PBS, and permeabilized with 0.1% Triton X-100 for 15 min at 4 °C. After washing with PBS, the cells were blocked with PBS containing 3% BSA for 1 h at room temperature and immunostained with anti-VE-cadherin (Santa Cruz Biotechnology, Santa Cruz, CA) and sheep anti-human Clec14a (R&D Systems, Minneapolis, MN) antibodies for 2 h at room temperature. The eyes of postnatal day 12 (P12) mouse pups were enucleated, and the retinas were dissected, fixed with paraformaldehyde for 3 h, and placed overnight in 70% ethanol, stained with TRITC-labeled *Griffonia simplicifolia* isolectin B4 (Invitrogen, Carlsbad, CA). The retinas were co-immunostained with mouse anti-human Clec14a antibodies (Abcam, Cambridge, UK). Proteins reacting with antibodies were visualized with species-matched Alexa 488-labeled or Alexa 546-labeled secondary antibodies. Fluorescence images of Alexa 488 and Alexa 546 were recorded with an Olympus IX-81 inverted fluorescence microscope (Olympus Corporation, Tokyo, Japan). Time-lapse images of HUVECs transfected with plasmids expressing green fluorescent protein (GFP) were obtained by an Olympus IX-81 inverted fluorescence microscope.

2.4. In vitro tube formation and in vitro wound-healing assay

Tube formation was assayed as previously described [16]. In brief, 250 μl Matrigel (BD Biosciences, Bedford, MA) was added to a 24-well plate and allowed to polymerize for 20 min at 37 °C. HUVECs were incubated in Medium 199 containing 20% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, 3 ng/mL basic fibroblast growth factor (Upstate Biotechnology, Waltham, MA), and 5 U/mL heparin. HUVECs were then harvested, resuspended in M199, and seeded onto the Matrigel (1.5×10^5 cells/well). Matrigel cultures were incubated at 37 °C and photographed at various time points. The area covered by the tube network was determined with an optical imaging technique; images of the tubes were scanned into Adobe Photoshop (Adobe Systems, San Jose, CA) and quantified by ImageJ software (National Institutes of Health, Bethesda, MD).

The wound-healing assay was performed by scratching confluent HUVECs on 2% gelatin-coated glass-based dish with a micropipette tip. Images were captured at 0 and 8 h after wounding. For quantitative analysis, five fields per plate were photographed, and distances between front lines were measured with ImageJ. Time-lapse images of HUVECs transfected with siRNA (Dharmacon,

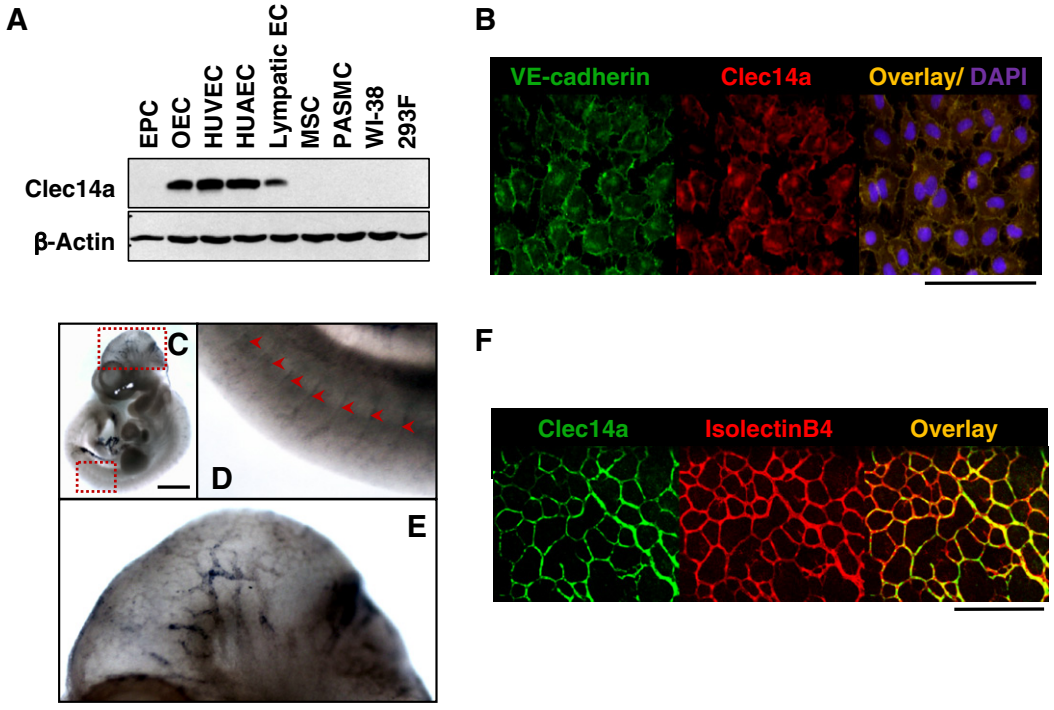

Fig. 1. Clec14a is specifically expressed in endothelial cell *in vitro* and *in vivo*. (A) Western blot analysis showing expression of Clec14a in human cell types. The expression of Clec14a appeared in outgrowth endothelial cells (OECs), human umbilical vein endothelial cells (HUVECs), human umbilical artery endothelial cells (HUAECs), and lymphatic endothelial cells (ECs). (B) Immunofluorescence images of anti-VE-cadherin (green) antibody, anti-Clec14a antibody (red), and DAPI staining (blue) in HUVECs. Clec14a colocalizes with VE-cadherin at the cell membrane. (C–E) Whole-mount *in situ* hybridization of Clec14a is showing strong expression at E10.5. Clec14a mRNA was detected in brain blood vessels and intersomitic blood vessels (arrowhead). (F) Immunofluorescence co-staining of anti-Clec14a and anti-isolectin B4 was detected in retina vessels of mouse pups at postnatal day 12. Scale bars are 100 μm in (B), 1 mm in (C), and 200 μm in (F).

Chicago, IL) against Clec14a were obtained by an Olympus IX-81 inverted fluorescence microscope.

2.5. Transfection siRNA

ECs were transfected with scrambled siRNA and Clec14a siRNA by the use of lipofectamin (Invitrogen, Carlsbad, CA) for 3 h. Cells were used for assays at 36 h after transfection. The siRNA of Clec14a was designed by Dharmacon Inc. For Clec14a, a siRNA with the sequence 5′-CAAUCAGGGUCGACGAGAA-3′ was used.

2.6. Cell aggregation assay

The 293F cell line was transfected with plasmids expressing GFP, Clec14a-GFP, Clec14aΔ^{cyto}-GFP, Clec14aΔ^{lectin}-GFP, Clec14a-Δ^{egf}-GFP, suspended in FreeStyle 293 expression (Invitrogen, Carlsbad, CA) medium, and seeded into 6-well plates (5.0×10^5 cells/well). The cell aggregation assay was performed as described previously [17]. The numbers of cell aggregates (>4 cells) were counted in at least 10 different fields.

2.7. Antibody generation

Polyclonal rabbit anti-mouse antibodies were generated against Clec14a. The antigen sequence of mClec14a corresponding to amino acids 123–136 was designed by GenScript Corporation (Piscataway, NJ) and the immunogen was conjugated to the carrier protein KLH. This polyclonal antibody was purified by affinity column.

3. Results

3.1. Clec14a is specifically expressed in the vasculature

We previously performed microchip array analysis to isolate genes specifically regulated during endothelial progenitor cell differentiation (GEO Accession No. GSE12891) [18], and identified Clec14a as one of genes upregulated during this process. To identify the cells in which it is expressed, we measured Clec14a mRNA levels in several human cell types (Supplementary Fig. 1A). In addition, we evaluated Clec14a protein expression in mouse tissues and different types of human cells (Supplementary Fig. 1B and Fig. 1A). Clec14a transcripts were detected in ECs, but not in other cells. Immunostaining of ECs revealed that Clec14a is expressed in the membrane region where VE-cadherin expression is detected (Fig. 1B). We also analyzed Clec14a expression in mouse embryos at day 10.5 (E10.5) by *in situ* hybridization. Clec14a was detected in the vasculature including intersomitic vessels and blood vessels of the brain in E10.5 embryos (Fig. 1C–E). Vessel-specific expression of Clec14a was also observed in the mouse retina at P12 (Fig. 1F). These results demonstrate that Clec14a is specifically expressed in vascular ECs.

3.2. Clec14a mediates cell–cell adhesion through the C-type lectin domain

To further characterize the role of Clec14a in cell–cell contact, we performed time-lapse imaging of ECs over-expressing the Clec14a carboxy-terminal fused with GFP (Clec14a^{FL}-GFP;

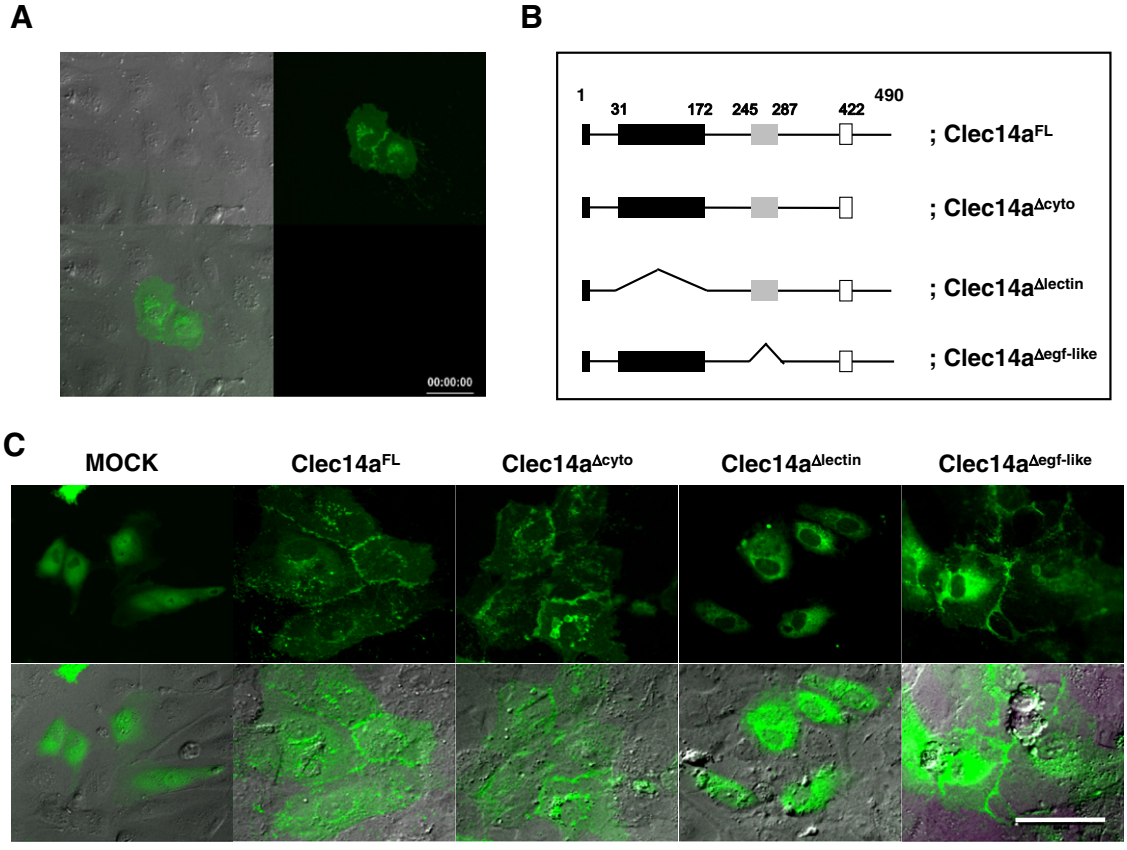

Fig. 2. The C-type lectin domain of Clec14a is required for endothelial cell–cell contact. (A) In this image from a time-lapse movie, Clec14a was detected in the region of cell–cell contacts. (B) Constructs generated by deletion mutagenesis of Clec14a. (C) In confluent HUVECs transfected with the deletion construct Clec14aΔ^{lectin}-GFP, green fluorescent protein (GFP) expression was not detected in the region of cell–cell contact, whereas the GFP expression of the other constructs was visible in that region. Scale bars are 100 μm in (A, C).

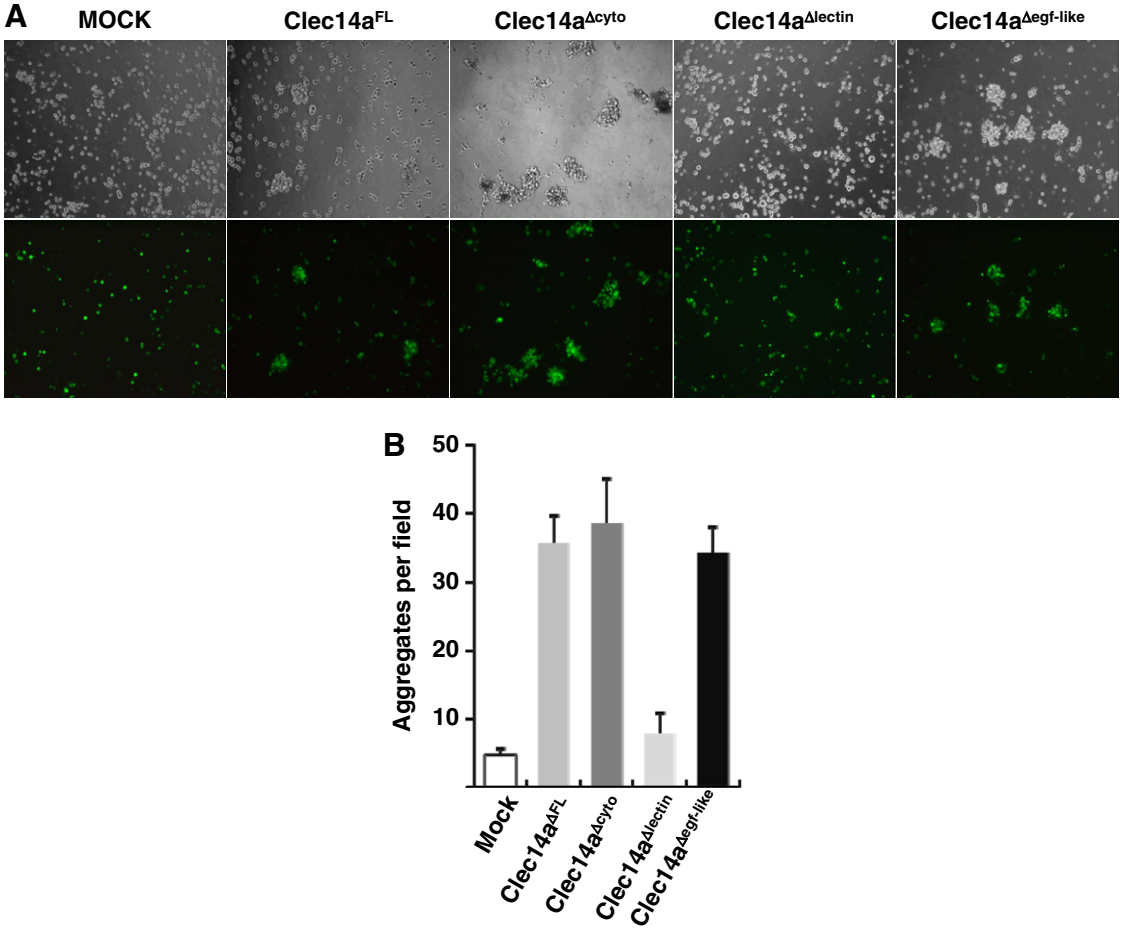

Fig. 3. Aggregation assay confirms that cell–cell adhesion is mediated by the C-type lectin domain. (A) Aggregation of 293F cells in suspension expressing GFP, Clec14a^{FL}-GFP, Clec14aΔ^{cyto}-GFP, Clec14aΔ^{lectin}-GFP, and Clec14aΔ$^{egf\text{-}like}$-GFP (phase-contrast image: upper panels, fluorescence image: lower panels). (B) To quantify aggregation, the number of cell aggregates (cell mass >4 cells) was counted.

Supplementary Movie 1 and Fig. 2A). Clec14a^{FL}-GFP appeared at the cell junctional region of contacting HUVECs. To determine the region of Clec14a responsible for the cell–cell interaction, we constructed a series of deletion mutants: Clec14aΔ^{cyto}-GFP, Clec14aΔ^{lectin}-GFP, and Clec14aΔ^{egf}-GFP (Fig. 2B). Interestingly, localization of Clec14a in the junctional region was significantly reduced in HUVECs overexpressing the C-type lectin domain deletion mutant (Clec14aΔ^{lectin}-GFP), whereas the localization of other mutants was similar to that of Clec14a^{FL}-GFP (Fig. 2C). To determine whether Clec14a mediates cell–cell adhesion, we performed a cell aggregation assay with 293F cells in suspension. Clec14a^{FL}-GFP-expressing 293F cells aggregated well, and Clec14aΔ^{cyto}-GFP and Clec14aΔ^{egf}-GFP expression also induced cell aggregation (Fig. 3A and B). However, aggregation was significantly decreased in cells expressing the C-type lectin domain deletion construct. These results suggest that the C-type lectin domain of Clec14a is involved in mediating cell–cell adhesion.

3.3. Silencing Clec14a impairs EC angiogenic activity

To determine the potential role of Clec14a in endothelial function, we reduced its expression by transfection with siRNA against human Clec14a (Fig. 4A). Clec14a knockdown delayed formation of EC tubular networks on Matrigel (Fig. 4B and C). Filopodia number in Clec14a-deficient ECs was also decreased compared to cells transfected with scrambled siRNA at 1 or 5 h after plating on Matrigel (Fig. 4D). Migratory activity of ECs is a critical feature of angiogenesis. The wound healing assay revealed that Clec14a knockdown decreased the migration distance of cells at the wound edge (Fig. 4E and F). Taken together, these findings demonstrate that Clec14a plays a critical role in the angiogenic activity of ECs.

4. Discussion

The thrombomodulin (TM) protein family, a subfamily of the C-type lectin superfamily, includes TM, endosialin, CD93, and Clec14a [1]. Although these proteins are involved in vascular activity, their expression is not limited to ECs. TM, a potent anticoagulant originally identified in ECs, is expressed not only in the vasculature as but also in the meninges, mesothelial cells, dermal keratinocytes, and platelets. CD93, a phagocytosis receptor, is expressed in myeloid lineage cells, ECs, platelets, and microglia, although several reports suggest it is predominantly expressed in ECs [12,19]. Endosialin/TEM1 is selectively expressed in tumor vascular endothelium [20] and has therefore been targeted therapeutically. The new member of the TM family, Clec14a, has not yet been characterized. In the present study, we demonstrated the selective expression of Clec14a in ECs. The endogenous expression of Clec14a was first detected in the developing vasculature of mice, which suggests that Clec14a may play an important role in vessel development.

The localization of Clec14a in the intercellular boundary region led us to examine its role in cell–cell adhesion. Overexpression of Clec14a-GFP in 293F cells induced aggregation, which was

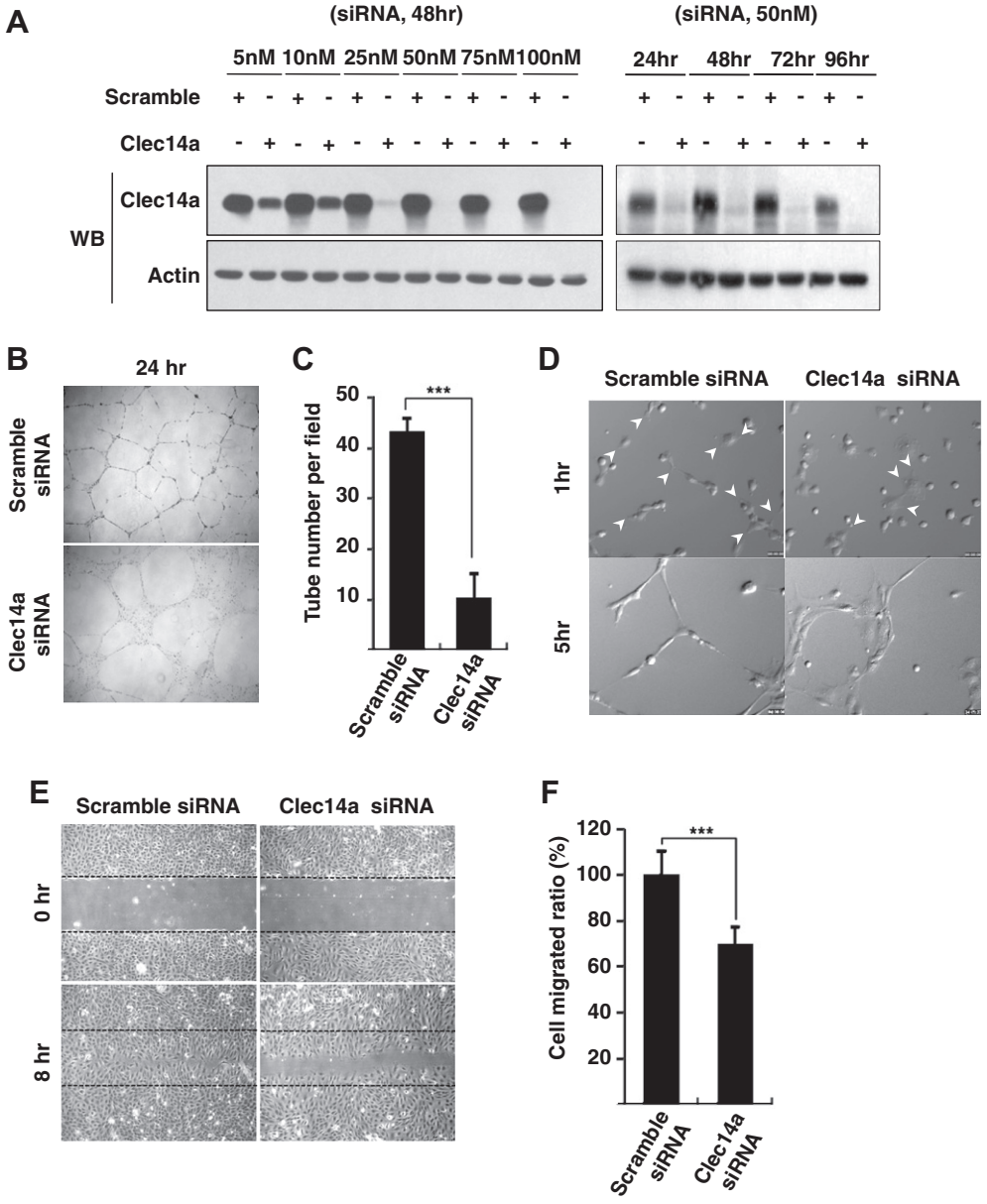

Fig. 4. Clec14a siRNA changed morphology and inhibited migration of human umbilical vein endothelial cells. (A) Down-regulation of Clec14a by siRNA is concentration-dependent and time-dependent. Human umbilical vein endothelial cells (HUVECs) were transfected with Clec14a siRNA, and tubular network formation on Matrigel of (B) confluent and (D) sparse cultures was photographed and (C) quantified. ***$P < .001$ compared with scrambled siRNA control group. (E) Endothelial cell (EC) migration after siRNA transfection as assessed by the wound-healing assay and (F) quantified. ***$P < .001$ compared with scrambled siRNA control group.

disrupted by deletion of its C-type lectin-like domain. Like other TM proteins, the extracellular region of Clec14a is composed of a C-type lectin-like domain, an EGF domain, and a sushi-like domain. Although deletion of the other domains did not reduce cell aggregation, C-type lectin domain was required for cell–cell interaction. A previous report showed that TM also mediates cell–cell adhesion, and specific binding of the C-type lectin-like domain to carbohydrates is responsible for these interactions [21]. Similarly, it is likely that Clec14a mediates cell adhesion via interaction of C-type lectin domain with carbohydrate-containing molecules, although the physiological ligands for Clec14a have not yet been determined. The physiological significance of Clec14a-mediated cell–cell adhesion also requires further investigation *in vivo*.

The potential involvement of Clec14a in angiogenesis was investigated in the present study using the *in vitro* assays of tube formation and migration of ECs. Knockdown of Clec14a limited the ability of HUVECs to organize into tubular structures on Matrigel, suggesting a role for Clec14a in *in vitro* endothelial tube formation. Vascular tube formation requires that ECs of the newly formed channel are stably associated. The concentration of Clec14a proteins at junctions between ECs and the induction of cell aggregation by Clec14a suggest that Clec14a participates in the formation of intercellular structures critical for stable vascular tubes. However, *in vitro* tube formation not only requires the ability to form stable cell–cell contacts, it also depends on EC motility and the ability to change shape before association. Results of the wound-induced migration assay demonstrated that Clec14a knockdown blocks HUVEC motility. These data suggest a role for Clec14a in EC motility, independent of its effects on EC-EC association. Interestingly, we also found that Clec14 is involved in EC adhesion to extracellular matrix (ECM; data not shown), which may account for the effect of Clec14a on EC migration. Endosialin has also been reported to mediate migration and cell adhesion to the ECM by interacting with fibronectin and collagen types I and

IV [22]. Taken together, our results indicate that Clec14a may play a critical role in the angiogenic function of ECs through interaction with other ECs or ECM proteins.

In conclusion, we have demonstrated that Clec14a may be an endothelial adhesion molecule involved in multiple functions. Because endothelial cell–cell interaction is important for stable vessel tube formation, it is worthwhile to investigate its role in leukocyte infiltration and vascular permeability, which is intimately associated with inflammatory angiogenesis. The functional role of Clec14a in the vasculature development has yet to be identified. Studies to clarify these issues are now underway.

Acknowledgments

This work was supported by a National Research Laboratory Grant (20100018854) and the Korea Biotech R&D Group of Next-generation growth engine project (2010K0012733-08A0301-00310) from the Ministry of Education and Science Technology, and a grant from the Korea Health 21 R&D Project, Ministry of Health Welfare & Family Affairs (A085136), Korea.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.11.075.

References

[1] A.N. Zelensky, J.E. Gready, C-type lectin-like domains in Fugu rubripes, BMC Genomics 5 (2004) 51.
[2] A.N. Zelensky, J.E. Gready, The C-type lectin-like domain superfamily, FEBS J. 272 (2005) 6179–6217.
[3] A. Cambi, C. Figdor, Necrosis: C-type lectins sense cell death, Curr. Biol. 19 (2009) R375–R378.
[4] K. Drickamer, C-type lectin-like domains, Curr. Opin. Struct. Biol. 9 (1999) 585–590.
[5] B. Isermann, S.B. Hendrickson, M. Zogg, M. Wing, M. Cummiskey, Y.Y. Kisanuki, M. Yanagisawa, H. Weiler, Endothelium-specific loss of murine thrombomodulin disrupts the protein C anticoagulant pathway and causes juvenile-onset thrombosis, J. Clin. Invest. 108 (2001) 537–546.
[6] E.M. Conway, M. Van de Wouwer, S. Pollefeyt, K. Jurk, H. Van Aken, A. De Vriese, J.I. Weitz, H. Weiler, P.W. Hellings, P. Schaeffer, J.M. Herbert, D. Collen, G. Theilmeier, The lectin-like domain of thrombomodulin confers protection from neutrophil-mediated tissue damage by suppressing adhesion molecule expression via nuclear factor kappaB and mitogen-activated protein kinase pathways, J. Exp. Med. 196 (2002) 565–577.
[7] C.S. Shi, G.Y. Shi, S.M. Hsiao, Y.C. Kao, K.L. Kuo, C.Y. Ma, C.H. Kuo, B.I. Chang, C.F. Chang, C.H. Lin, C.H. Wong, H.L. Wu, Lectin-like domain of thrombomodulin binds to its specific ligand Lewis Y antigen and neutralizes lipopolysaccharide-induced inflammatory response, Blood 112 (2008) 3661–3670.
[8] A. Nanda, B. Karim, Z. Peng, G. Liu, W. Qiu, C. Gan, B. Vogelstein, B. St Croix, K.W. Kinzler, D.L. Huso, Tumor endothelial marker 1 (Tem1) functions in the growth and progression of abdominal tumors, Proc. Natl. Acad. Sci. USA 103 (2006) 3351–3356.
[9] W.J. Rettig, P. Garin-Chesa, J.H. Healey, S.L. Su, E.A. Jaffe, L.J. Old, Identification of endosialin, a cell surface glycoprotein of vascular endothelial cells in human cancer, Proc. Natl. Acad. Sci. USA 89 (1992) 10832–10836.
[10] B. Tomkowicz, K. Rybinski, D. Sebeck, P. Sass, N.C. Nicolaides, L. Grasso, Y. Zhou, Endosialin/TEM-1/CD248 regulates pericyte proliferation through PDGF receptor signaling, Cancer Biol. Ther. 9 (2010) 908–915.
[11] N. Simonavicius, D. Robertson, D.A. Bax, C. Jones, I.J. Huijbers, C.M. Isacke, Endosialin (CD248) is a marker of tumor-associated pericytes in high-grade glioma, Mod. Pathol. 21 (2008) 308–315.
[12] P.J. Norsworthy, L. Fossati-Jimack, J. Cortes-Hernandez, P.R. Taylor, A.E. Bygrave, R.D. Thompson, S. Nourshargh, M.J. Walport, M. Botto, Murine CD93 (C1qRp) contributes to the removal of apoptotic cells in vivo but is not required for C1q-mediated enhancement of phagocytosis, J. Immunol. 172 (2004) 3406–3414.
[13] V. Marin, G. Kaplanski, S. Gres, C. Farnarier, P. Bongrand, Endothelial cell culture: protocol to obtain and cultivate human umbilical endothelial cells, J. Immunol. Methods 254 (2001) 183–190.
[14] M.A. Nieto, K. Patel, D.G. Wilkinson, In situ hybridization analysis of chick embryos in whole mount and tissue sections, Methods Cell Biol. 51 (1996) 219–235.
[15] N. Schaeren-Wiemers, A. Gerfin-Moser, A single protocol to detect transcripts of various types and expression levels in neural tissue and cultured cells: in situ hybridization using digoxigenin-labelled cRNA probes, Histochemistry 100 (1993) 431–440.
[16] J.K. Min, Y.L. Cho, J.H. Choi, Y. Kim, J.H. Kim, Y.S. Yu, J. Rho, N. Mochizuki, Y.M. Kim, G.T. Oh, Y.G. Kwon, Receptor activator of nuclear factor (NF)-kappaB ligand (RANKL) increases vascular permeability: impaired permeability and angiogenesis in eNOS-deficient mice, Blood 109 (2007) 1495–1502.
[17] S. Fukuhara, K. Sako, T. Minami, K. Noda, H.Z. Kim, T. Kodama, M. Shibuya, N. Takakura, G.Y. Koh, N. Mochizuki, Differential function of Tie2 at cell–cell contacts and cell-substratum contacts regulated by angiopoietin-1, Nat. Cell Biol. 10 (2008) 513–526.
[18] Y.S. Maeng, H.J. Choi, J.Y. Kwon, Y.W. Park, K.S. Choi, J.K. Min, Y.H. Kim, P.G. Suh, K.S. Kang, M.H. Won, Y.M. Kim, Y.G. Kwon, Endothelial progenitor cell homing: prominent role of the IGF2–IGF2R–PLCbeta2 axis, Blood 113 (2009) 233–243.
[19] E.P. McGreal, N. Ikewaki, H. Akatsu, B.P. Morgan, P. Gasque, Human C1qRp is identical with CD93 and the mNI-11 antigen but does not bind C1q, J. Immunol. 168 (2002) 5222–5232.
[20] B. St Croix, C. Rago, V. Velculescu, G. Traverso, K.E. Romans, E. Montgomery, A. Lal, G.J. Riggins, C. Lengauer, B. Vogelstein, K.W. Kinzler, Genes expressed in human tumor endothelium, Science 289 (2000) 1197–1202.
[21] H.C. Huang, G.Y. Shi, S.J. Jiang, C.S. Shi, C.M. Wu, H.Y. Yang, H.L. Wu, Thrombomodulin-mediated cell adhesion: involvement of its lectin-like domain, J. Biol. Chem. 278 (2003) 46750–46759.
[22] B. Tomkowicz, K. Rybinski, B. Foley, W. Ebel, B. Kline, E. Routhier, P. Sass, N.C. Nicolaides, L. Grasso, Y. Zhou, Interaction of endosialin/TEM1 with extracellular matrix proteins mediates cell adhesion and migration, Proc. Natl. Acad. Sci. USA 104 (2007) 17965–17970.