Biochemical and Biophysical Research Communications 464 (2015) 590–595

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

VEGF-induced blood flow increase causes vascular hyper-permeability *in vivo*

Kohei Ashina, Yoshiki Tsubosaka, Koji Kobayashi, Keisuke Omori, Takahisa Murata*

Department of Animal Radiology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan

ARTICLE INFO

Article history:
Received 30 June 2015
Accepted 2 July 2015
Available online 7 July 2015

Keywords:
VEGF
Vascular permeability
Blood flow
Endothelial barrier
Nitric oxide

ABSTRACT

VEGF is known to cause vascular leak, its detailed mechanisms *in vivo* remain unclear. Here, we investigated the mechanisms underlying VEGF-induced vascular hyper-permeability focusing on two major regulators of vascular permeability: blood flow and endothelial barrier function. Administration of VEGF caused vascular hyper-permeability and tissue swelling in mouse ears, which were abolished by VEGF receptor-2 blockade. Intravital imaging showed that VEGF dilated ear arteries but not veins, and laser Doppler velocimetry showed that VEGF quickly increased tissue blood flow along with arterial dilation. Whole-mount immunostaining showed that VEGF phosphorylated endothelial nitric oxide synthase (eNOS) at residue Ser1177 and disrupted the alignment of vascular endothelial-cadherin (VE-cadherin) around the endothelial cell borders in mouse ear skin, indicating endothelial nitric oxide (NO) production and barrier disruption. Administration of the nitric oxide synthesis inhibitor, L-NAME, as well as the vasoconstrictor phenylephrine, abolished all VEGF-induced responses, including blood flow increase, dye leakage, and tissue swelling. However, these two treatments did not alter the intracellular localization of VE-cadherin-induced by VEGF. These observations underscore the importance of vascular dilation and, subsequent increase in blood flow, as well as, endothelial barrier disruption in the mechanisms of VEGF-induced vascular hyper-permeability.

1. Introduction

The vasculature that runs through the human body plays an important role in maintaining tissue homeostasis by supplying nutrients and oxygen [1], with vascular endothelial and mural cells, being the two major cell types. An endothelial cell monolayer covers the inner surface of blood vessels, and several layers of mural cells, including vascular smooth muscle cells and pericytes, line the outside of the endothelial layer. Vascular endothelial cells and smooth muscle cells functionally coordinate a variety of vascular functions such as the regulation of vascular tone and vascular permeability.

Vascular permeability plays a crucial role in the regulation of leukocyte infiltration and leakage of plasma components to maintain tissue homeostasis [1]. Upon inflammation, the secretion of inflammatory cytokines by damaged tissue resident cells and/or infiltrating immune cells, renders the vasculature hyper-permeable and leads to the subsequent leak of leukocytes and plasma components into the interstitium. Although these processes are essential to protect tissues against infection or physical damage [2], excessive and/or persistent vascular hyper-permeability causes tissue edema, and in some cases leads to further disease progressions. Indeed, vascular hyper-permeability is thought to be an exacerbating factor of acute lung injury, and solid tumors [3]. Thus, modulators of vascular permeability are being investigated as potential drug targets.

Vascular permeability *in vivo* is thought to be regulated mainly by blood flow and endothelial barrier function. A clinical study showed that administration of the vasoconstrictor phenylephrine abolished rhinorrhea in allergic rhinitis [4]. By contrast, an *in vivo* experimental study showed that administration of the vasodilator bradykinin increased blood flow and subsequently caused vascular hyper-permeability in hamster cheek pouch vessels [5]. Moreover, we previously reported that the inflammatory mediator, prostaglandin E_2 increased vascular permeability, an effect dependent on

Abbreviations: eNOS, endothelial nitric oxide synthase; NO, nitric oxide; VE-cadherin, vascular endothelial-cadherin; VEGF, VEGF-A.

* Corresponding author. Department of Animal Radiology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan.
E-mail address: amurata@mail.ecc.u-tokyo.ac.jp (T. Murata).

http://dx.doi.org/10.1016/j.bbrc.2015.07.014
0006-291X/

vascular dilation and increased in blood flow [6]. These findings indicate that blood flow is a major determinant factor in the modulation of vascular permeability *in vivo*. However, only few studies demonstrating its functional contribution exist.

The endothelial barrier, constituted by the cell–cell adherens junction and composed of VE-cadherin, catenin, and cytoskeleton, is another important factor involved in the modulation of vascular permeability. Sphingosine-1-phosphate [7] or prostaglandin D_2 [8] tightens the adherens junction and enhances endothelial barrier properties by the activation of PI3K/Akt-signaling and cAMP/PKA-signaling, respectively. By contrast, thrombin disrupts endothelial barrier formation by activation of calcium/RhoA-signaling [9]. Moreover, platelet-activating factor also disrupts the endothelial barrier by activating Tiam1-Rac1 signaling [10]. Thus, although multiple *in vitro* studies on the mechanisms of endothelial barrier modulation have been carried out, its functional importance *in vivo* remains unclear.

VEGF-A (VEGF), also known as vascular permeability factor [11], was originally described as an endothelial cell-specific mitogen [12]. VEGF is produced by many cell types including tumor cells, macrophages, platelets, and keratinocytes, mainly in response to low oxygen stress [13]. Moreover, VEGF increases vascular permeability and leads to angiogenesis in physiological situations, such as bone formation, hematopoiesis, and wound healing, as well as in pathological conditions such as inflammatory diseases, diabetic retinopathy, and solid tumors [13]. Thus, the mechanisms involved in the regulation of VEGF-signaling under physiological/pathological conditions are the subject of active research aimed at the development of new therapies for VEGF-related diseases.

VEGF exerts its biological effects mainly through 3 primary receptors, VEGFR1, VEGFR2, and VEGFR3, with VEGFR-2 being the main receptor associated with vascular hyper-permeability and angiogenesis [14]. *In vitro* studies focusing on the adherens junction and cytoskeleton rearrangement in endothelial cells have revealed the mechanisms underlying VEGF-induced vascular endothelial hyper-permeability. Chen et al. reported that VEGF phosphorylates VE-cadherin, and β-catenin [15], whereas Gavard et al. showed that VEGF induces endocytosis of VE-cadherin [16]. Moreover, other investigators showed that VEGF activates eNOS, leading to NO production and cytoskeleton rearrangements [17], and nitrosylates β-catenin [18]. In addition, NO appeared to plays an important role in VEGF-induced vascular hyper-permeability. However, the mechanisms by which VEGF modulates vascular permeability *in vivo* remain unclear.

In this study, we attempted to reveal the mechanism of VEGF-induced vascular hyper-permeability focusing on blood flow and endothelial barrier function *in vivo*.

2. Materials and methods

2.1. Mice

All animal experiments were approved by the Iinstitutional Animal Care and Use Ethical Committee of the University of Tokyo (p11-578), and performed according to the National Institute of Health guidelines. FVB/NJcl mice (8 weeks old) were purchased from CLEA, Japan. To perform the experiments, general anesthesia was induced and maintained with 4% and 2% isoflurane, respectively, via a nose cone.

2.2. Miles assay

To assess vascular permeability, 50 mg/kg Evans blue (Sigma–Aldrich) was injected into the tail vein, 10 min after intradermal administration of VEGF-A165 (Wako). Ear thickness was measured 30 min after dye-injection, using slide calipers. The ear was then dissected, dried in a constant-temperature oven, and weighed. The extravasated Evans blue present in the ear was extracted in formamide, and quantified spectrophotometrically at a wavelength of 610 nm.

2.3. Intravital microscopy

To visualize mouse ear vessels, 70 kDa FITC-dextran (10 mg/kg in 100 μl phosphate-buffered saline, Sigma–Aldrich) was injected intravenously. Mice were then positioned on the microscope stage while maintaining their body temperatures at 37 °C, and VEGF was injected intradermally in the ear. The vascular diameter was monitored every 5 min and quantified using a confocal microscope (confocal system C1, Nikon).

2.4. Whole-mount immunostaining

Mice were euthanized and immediately fixed by perfusion with 4% paraformaldehyde. Their ears were then dissected and skinned. Skin samples were permeabilized with 0.15% Triton X-100, incubated with blocking buffer containing 5% normal donkey serum for 30 min, and probed with the following primary antibodies overnight at 4 °C: goat anti-VE-cadherin (Santa Cruz Biotechnology), rabbit anti-eNOS (Santa Cruz Biotechnology), rat anti-CD31 (Biocare Medical), or rabbit anti-phospho-eNOS (Ser1177, Cell Signaling). After incubation for 2 h at room temperature with the following secondary antibodies: Alexa Fluor 488 anti-goat IgG, Alexa Fluor 594 anti-rabbit IgG or Alexa Fluor 488 anti-rat IgG, the nuclei were labeled with DAPI (1 μg/ml) for 20 min prior to imaging by confocal microscopy.

2.5. Laser Doppler velocimetry measurements

VEGF was administered intradermally under anesthesia, and changes in mouse ear blood flow were monitored with an Omegazone laser Doppler blood-flow imaging system (Omegawave).

2.6. Reagents

The following reagents were obtained from the indicated suppliers: VEGF-A165, (Wako); L-phenylephrine hydrochloride (Sigma–Aldrich); histamine dihydrochloride, (Wako); L-NAME, (Enzo Life Sciences); LY294002 (Calbiochem); Ki8751 (Adooq Bioscience).

2.7. Statistical analyses

The results of the experiments were expressed as means ± standard error of the mean (SEM). Statistical evaluation of the data was performed using an unpaired Student's *t*-test or a one-way ANOVA, followed by the Tukey's multiple comparison test. A value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. VEGF increases vascular permeability via VEGFR2

Administration of VEGF (0.3–30 ng/ear, 10 min) induced dye extravasation and ear tissue swelling in a dose-dependent manner (representative pictures of dye extravasation are shown in Fig. 1A and the data are summarized in Fig. 1B and C). Pretreatment with a VEGFR2 inhibitor, Ki8751 (5 μg/ear, 15 min before VEGF administration) completely inhibited VEGF (30 ng/ear)-induced dye extravasation and tissue swelling (Fig. 1D and E).

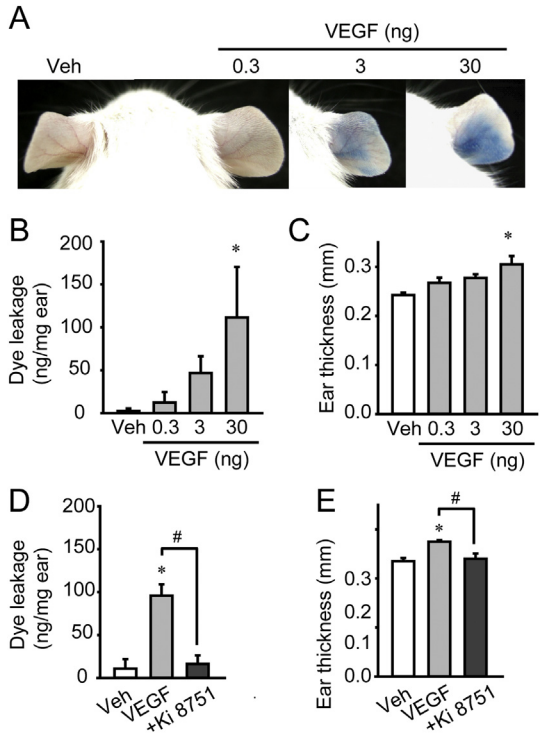

Fig. 1. VEGFR2 activation increased vascular permeability. Effects of VEGF on vascular hyper-permeability. (A) Photographs of representative mouse ears. (B) Quantification of Evans blue leakage (n = 4). (C) Quantification of ear thickness (n = 4). (D) Quantification of Evans blue leakage after Ki8751 exposure (n = 4). (E) Quantification of ear thickness after Ki8751 exposure (n = 4). *P < 0.05, compared with vehicle. #P < 0.05, compared with VEGF. Data are presented as mean ± SEM.

3.2. VEGF dilates arteries and increases blood flow

We next investigated how VEGF stimulation changes vascular properties using an intravital imaging. As shown in Fig. 2, VEGF (3–30 ng/ear) induced arterial dilation within 5 min (Fig. 2A), which decreased gradually within 10 min (Fig. 2B). The fluorescent dye that leaked out of the vascular wall was also observed within 5 min of the stimulation (Fig. 2A, arrow head indicates the leaked dye fluorescence). Interestingly, VEGF administration did not influence venous diameter throughout the experimental period (Fig. 2C). Pretreatment with the VEGFR2 inhibitor Ki8751 (5 μg/ear, 15 min before VEGF-stimulation), almost abolished VEGF-induced arterial dilation (Fig. 2D).

As arterial dilation decreases vascular resistance and results in blood flow increase, we next measured blood flow using laser Doppler velocimetry. Along with the vascular dilation mentioned above, VEGF administration (30 ng/ear) rapidly increased tissue blood flow within 5 min, followed by a gradual reduction within a 10-min period (Fig. 2E and F). These results suggested that VEGF dilates tissue arteries and increases local blood flow via VEGFR2 activation.

3.3. VEGF dilates skin arteries

We next attempted to assess the relationship between the increase in blood flow and vascular hyper-permeability after VEGF-stimulation. Administration of the NO inhibitor L-NAME (80 μg/ear, 15 min before the VEGF-stimulation) almost completely inhibited VEGF-induced dye leakage (representative pictures are shown in Fig. 3A, data are summarized in Fig. 3B) and ear tissue swelling (Fig. 3C). Similarly, phenylephrine-induced

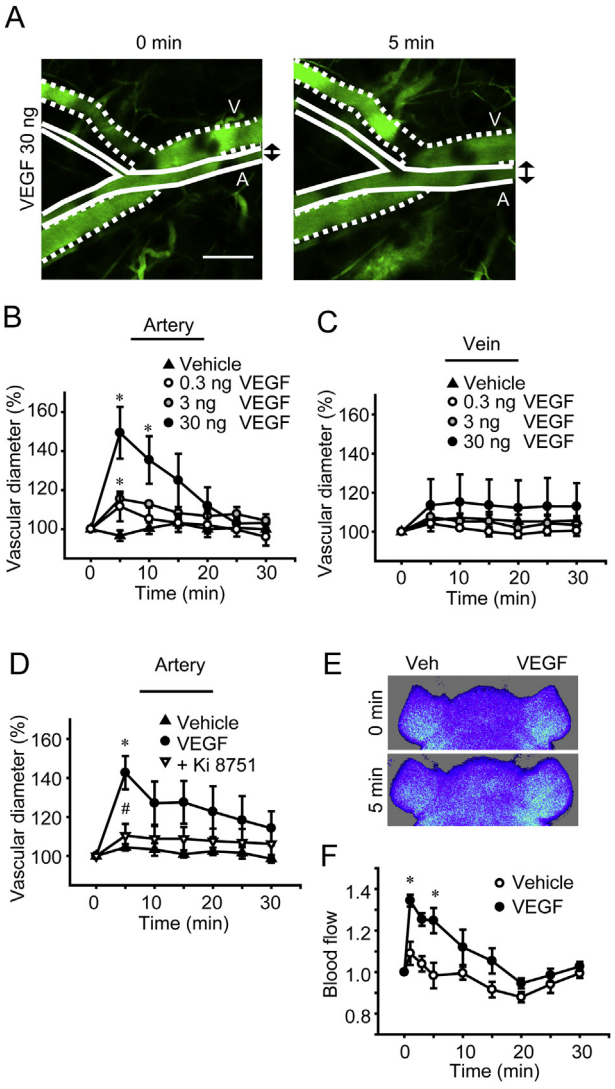

Fig. 2. VEGF induced arterial dilation and blood flow increase. (A) Typical images of VEGF-induced arterial dilation. Bar, 100 μm. A; artery, V; vein. (B, C) Quantification of the rate of vascular diameter change (B; artery, C; vein) (n = 4–5). (D) Effect of Ki8751 on VEGF-induced arterial dilation (n = 4–5). (E) Typical images of changes in local blood flow. (F) Quantification of changes in local blood flow (n = 4). *P < 0.05, compared with vehicle. #P < 0.05, compared with VEGF. Data are presented as mean ± SEM.

vasoconstriction (1 μg/ear, 15 min pretreatment) also inhibited VEGF-induced dye leakage (Fig. 3A and B) and ear tissue swelling (Fig. 3C).

As expected, intravital imaging showed that the administration of L-NAME (80 μg/ear, 15 min pretreatment) or phenylephrine (1 μg/ear, 15 min pretreatment) abolished VEGF-induced arterial dilation (Fig. 3D). These observations implied that VEGF-induced vascular hyper-permeability is mediated by NO-dependent arterial dilation.

3.4. VEGF phosphorylates eNOS via activation of PI3K-signaling

Previous studies revealed that VEGF phosphorylates eNOS at residue Ser1177 to produce NO through PI3K-signaling activation. We next investigated whether VEGF phosphorylates vascular endothelial cells eNOS *in vivo*. As shown in Fig. 4A, the endothelial cell marker PECAM-1 localized at the cell-to-cell junctions, whereas

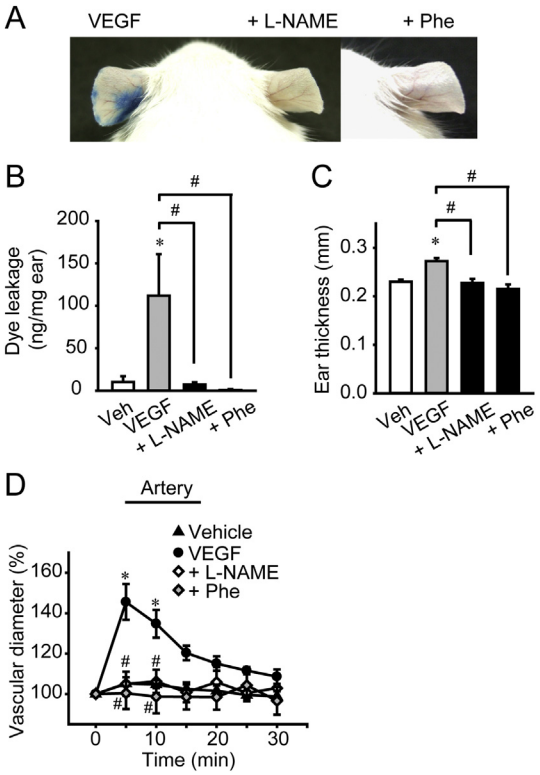

Fig. 3. Pretreatment with L-NAME or phenylephrine inhibited VEGF-induced hyper-permeability and vascular relaxation. (A) Effect of L-NAME or phenylephrine on VEGF-induced vascular hyper-permeability. Typical photographs showing extravasation of Evans blue after VEGF treatment. (B) Quantification of Evans blue leakage (n = 4). (C) Quantification of the ear thickness (n = 4). (D) Quantification of arterial diameter changes (n = 4–5). *P < 0.05, compared with vehicle. #P < 0.05, compared with VEGF. Data are presented as mean ± SEM.

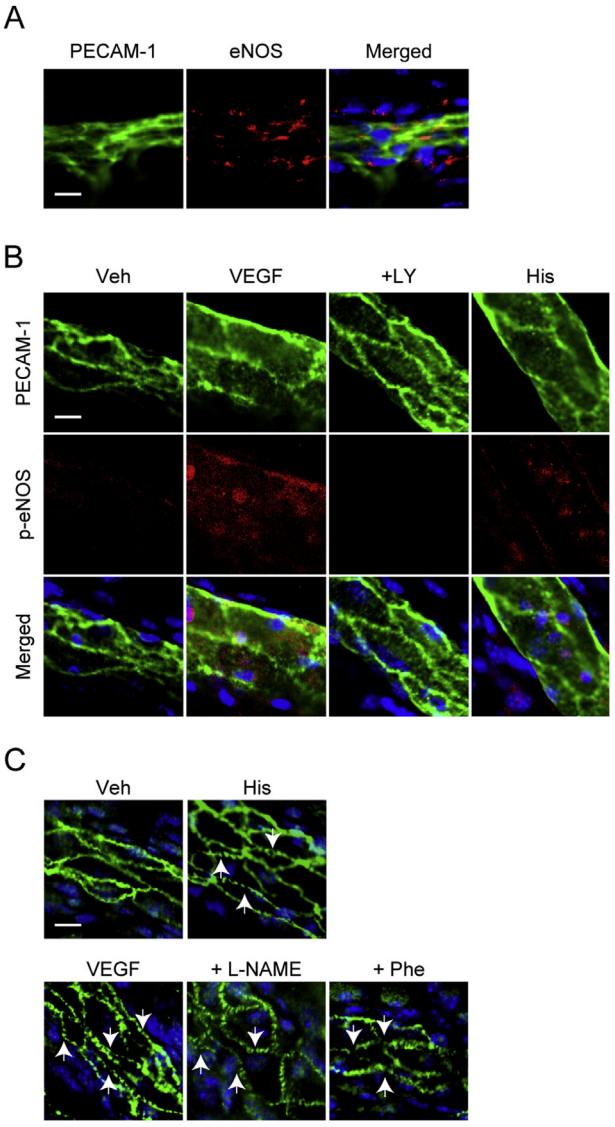

Fig. 4. VEGF phosphorylated eNOS and disrupted endothelial barrier integrity. (A) Whole-mount staining of PECAM-1 and eNOS in ear vascular endothelial cells. (B) Whole-mount staining of PECAM-1 and phospho-eNOS (Ser1177) in ear vascular endothelial cells. (C) Whole-mount staining of VE cadherin in ear vascular endothelial cells. Bar, 10 μm.

eNOS localized around the perinuclear areas in endothelial cells. As shown in Fig. 4B, phosphorylated eNOS was not observed in untreated cells, but it was detected 10 min after VEGF-stimulation (30 ng/ear, 10 min). Moreover, administration of the PI3K inhibitor LY294002 (5 ng/ear, 30 min before VEGF-stimulation) inhibited VEGF-induced eNOS-phosphorylation (Fig. 4B). In addition, histamine (0.4 mg/ear) also induced eNOS phosphorylation (Fig. 4B). These results support our conclusion that VEGF dilated ear arteries through eNOS phosphorylation-mediated NO production.

3.5. *VEGF disrupts endothelial barrier integrity*

Whole-mount immunostaining was performed to assess whether VEGF affects endothelial barrier formation in ear vessels. As shown in Fig. 4C, the green signal of VE-cadherin localized at the cell–cell junctions in ear vessels before stimulation (Fig. 4C). However, administration of VEGF (30 ng/ear, 10 min) disrupted VE-cadherin the alignment, suggesting endothelial barrier disruption (indicated by arrows). Notably, pretreatment with L-NAME (80 μg/ear) or phenylephrine for 15 min (1 μg/ear) did not influence VEGF-induced VE-cadherin alignment disruption (Fig. 4C). Moreover, administration of the potent hyper-permeability factor histamine (0.4 mg/ear) also disrupted the VE-cadherin alignment (Fig. 4C). These results suggest that VEGF disrupts the endothelial barrier in mouse ears.

4. Discussion

Understanding the underlying mechanisms involved in physiological responses associated with VEGF-induced vascular hyper-permeability is essential to provide insights into VEGF-related diseases and the development of novel therapies [19]. Although most studies have attempted to reveal the mechanism by which VEGF regulates endothelial barrier formation [15,17,20], comprehensive studies focusing on the effect of VEGF on both, blood flow change and endothelial barrier formation, are still needed. In this study, we showed that VEGF-induced hyper-permeability is accompanied by an increase in blood flow and a disruption of endothelial barrier formation *in vivo*.

NO synthesis inhibition or vasoconstriction abolished the increase in blood flow (Fig. 2E and F) and vascular hyper-permeability induced by VEGF (Fig. 1A–C) without affecting endothelial barrier integrity (Fig. 4C). We previously reported that PGE_2 induces vascular hyper-permeability through vessel dilation and increased local blood flow, while tightening the endothelial barrier integrity *in vivo* [21]. However, results from other studies suggest that an increase in blood flow leads to vascular hyper-permeability through the alteration of shear stress and hydrostatic fluid pressure. In fact, Orsenigo et al. showed that increased shear stress accompanied by

an increase in blood flow phosphorylates VE-cadherin and disrupts the endothelial barrier in mice, while Curry et al. suggested that elevation in intravascular pressure caused by blood flow increase leads to the leakage of plasma components into the extravascular area [22]. Although it is difficult to dissociate clearly the contributions of blood flow and endothelial barrier integrity *in vivo*, these observations highlight the role of blood flow change in the modulation of vascular permeability.

Vessel dilation leads to a local increase in blood flow, and we showed on intravital imaging, that VEGF stimulation quickly dilated skin arteries in mouse ears (Fig. 2A and B). In line with our observations, several groups have reported VEGF-induced vascular relaxations. While Ku et al. originally showed that VEGF induces NO-dependent relaxation of isolated canine coronary arteries within 5 min of exposure [23], other investigators have reported that VEGF-induces vascular relaxation in rat aorta [24], human internal mammary artery [25] and pig skin vasculature [26]. Altogether, these results indicated that VEGF increases local blood flow through NO-dependent relaxation of skin arteries.

Endothelial barrier function is also a major key regulator of vascular permeability, and several inflammatory mediators, such as platelet-activating factor [10] and thrombin, increase vascular permeability by disrupting the endothelial barrier. Moreover, numerous studies have shown that VEGF disrupts the endothelial barrier *in vivo* and *in vitro*. Esser et al. reported that VEGF disrupts the endothelial barrier by phosphorylating VE-cadherin in human umbilical vein endothelial cells [20], while Chen et al. showed that phosphorylation of β-catenin induced by VEGF dissociates the VE-cadherin/catenin complex, resulting in endothelial barrier disruption in mice [15]. Consistent with these observations, we showed that VEGF disrupts endothelial integrity in ear vessels. Thus, endothelial barrier disruption is presumably a crucial factor in VEGF-induced vascular hyper-permeability. Furthermore, NO inhibition or vascular constriction restricted VEGF-induced vascular hyper-permeability, and disrupted the endothelial adherence junction. Therefore, endothelial barrier formation is likely to be a determinant factor of vascular leakage, which lays downstream of the increased in blood flow induced by VEGF stimulation.

NO is also a known regulator of endothelial barrier integrity. NO-induced phosphorylation of VE-cadherin disrupts endothelial barrier in human dermal microvascular endothelial cells [17], and NO-induced S-nitrosylation of β-catenin has a similar effect in bovine aortic endothelial cells [18]. In this study, we showed that VEGF increases eNOS phosphorylation at residue Ser1177 in mouse ears, indicating NO production. However, eNOS inhibition did not block VEGF-induced endothelial barrier disruption despite inhibiting vasodilation and blood flow increase (Figs. 3D and 4C). Therefore, whether or not VEGF-induced NO production disrupts endothelial barrier integrity in mouse ear vessels remains unclear.

The contractility of vascular mural cell modulates local blood flow, and we showed that vascular mural cells contribute to local vascular permeability in mouse ear skin. However, vascular structure varies depending on the type and/or site of blood vessels. For example, in the skin, many vessels are composed of endothelial cells and mural cells, i.e., smooth muscle cells and pericytes, but in the lung, microvessels are mainly composed of endothelial cells. In solid tumors, blood vessels appear as very immature structures with discontinuous endothelial-cell lining and poor coverage of smooth muscle cells [27]. Since each tissue seems to have typical vascular structures and systems to modulate vascular permeability, detailed studies using a variety of tissues are necessary.

In this study, we revealed that VEGF increases vascular permeability by increasing blood flow and disrupting the endothelial barrier *in vivo*. These findings will provide the basis for new therapeutic insights into VEGF-related diseases.

Acknowledgments

This work was supported by the Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry (26029A); a Grant-in-Aid from the Japan Society for the Promotion of Science (25252049 and 26660225); the SENSHIN Medical Research Foundation; Life Science Foundation of Japan; ONO Medical Research Foundation; the Takeda Science Foundation; the Foundation for Dietary Scientific Research.

Conflicts of interest

None.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.07.014.

References

[1] D. Mehta, A.B. Malik, Signaling mechanisms regulating endothelial permeability, Physiol. Rev. 86 (2006) 279–367.
[2] K. Ley, C. Laudanna, M.I. Cybulsky, S. Nourshargh, Getting to the site of inflammation: the leukocyte adhesion cascade updated, Nat. Rev. Immunol. 7 (2007) 678–689.
[3] S.M. Weis, Vascular permeability in cardiovascular disease and cancer, Curr. Opin. Hematol. 15 (2008) 243–249.
[4] R.A. Nathan, The pathophysiology, clinical impact, and management of nasal congestion in allergic rhinitis, Clin. Ther. 30 (2008) 573–586.
[5] M. Feletou, E. Bonnardel, E. Canet, Bradykinin and changes in microvascular permeability in the hamster cheek pouch: role of nitric oxide, Br. J. Pharmacol. 118 (1996) 1371–1376.
[6] K. Omori, T. Kida, M. Hori, H. Ozaki, T. Murata, Multiple roles of the PGE2 -EP receptor signal in vascular permeability, Br. J. Pharmacol. 171 (2014) 4879–4889.
[7] J.G. Garcia, F. Liu, A.D. Verin, A. Birukova, M.A. Dechert, W.T. Gerthoffer, J.R. Bamberg, D. English, Sphingosine 1-phosphate promotes endothelial cell barrier integrity by Edg-dependent cytoskeletal rearrangement, J. Clin. Invest. 108 (2001) 689–701.
[8] K. Kobayashi, Y. Tsubosaka, M. Hori, S. Narumiya, H. Ozaki, T. Murata, Prostaglandin D2-DP signaling promotes endothelial barrier function via the cAMP/PKA/Tiam1/Rac1 pathway, Arterioscler. Thromb. Vasc. Biol. 33 (2013) 565–571.
[9] G.P. van Nieuw Amerongen, S. van Delft, M.A. Vermeer, J.G. Collard, V.W. van Hinsbergh, Activation of RhoA by thrombin in endothelial hyperpermeability: role of Rho kinase and protein tyrosine kinases, Circulation Res. 87 (2000) 335–340.
[10] I.I. Knezevic, S.A. Predescu, R.F. Neamu, M.S. Gorovoy, N.M. Knezevic, C. Easington, A.B. Malik, D.N. Predescu, Tiam1 and Rac1 are required for platelet-activating factor-induced endothelial junctional disassembly and increase in vascular permeability, J. Biol. Chem. 284 (2009) 5381–5394.
[11] D.R. Senger, S.J. Galli, A.M. Dvorak, C.A. Perruzzi, V.S. Harvey, H.F. Dvorak, Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid, Science 219 (1983) 983–985.
[12] N. Ferrara, W.J. Henzel, Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells, Biochem. Biophys. Res. Commun. 161 (1989) 851–858.
[13] A. Hoeben, B. Landuyt, M.S. Highley, H. Wildiers, A.T. Van Oosterom, E.A. De Bruijn, Vascular endothelial growth factor and angiogenesis, Pharmacol. Rev. 56 (2004) 549–580.
[14] S. Koch, S. Tugues, X. Li, L. Gualandi, L. Claesson-Welsh, Signal transduction by vascular endothelial growth factor receptors, Biochem. J. 437 (2011) 169–183.
[15] X.L. Chen, J.O. Nam, C. Jean, C. Lawson, C.T. Walsh, E. Goka, S.T. Lim, A. Tomar, I. Tancioni, S. Uryu, J.L. Guan, L.M. Acevedo, S.M. Weis, D.A. Cheresh, D.D. Schlaepfer, VEGF-induced vascular permeability is mediated by FAK, Dev. Cell 22 (2012) 146–157.
[16] J. Gavard, J.S. Gutkind, VEGF controls endothelial-cell permeability by promoting the beta-arrestin-dependent endocytosis of VE-cadherin, Nat. Cell Biol. 8 (2006) 1223–1234.
[17] A. Di Lorenzo, M.I. Lin, T. Murata, S. Landskroner-Eiger, M. Schleicher, M. Kothiya, Y. Iwakiri, J. Yu, P.L. Huang, W.C. Sessa, eNOS-derived nitric oxide regulates endothelial barrier function through VE-cadherin and Rho GTPases, J. Cell Sci. 126 (2013) 5541–5552.
[18] S. Thibeault, Y. Rautureau, M. Oubaha, D. Faubert, B.C. Wilkes, C. Delisle, J.P. Gratton, S-nitrosylation of beta-catenin by eNOS-derived NO promotes VEGF-induced endothelial cell permeability, Mol. Cell 39 (2010) 468–476.

[19] S.M. Weis, D.A. Cheresh, Pathophysiological consequences of VEGF-induced vascular permeability, Nature 437 (2005) 497–504.
[20] S. Esser, M.G. Lampugnani, M. Corada, E. Dejana, W. Risau, Vascular endothelial growth factor induces VE-cadherin tyrosine phosphorylation in endothelial cells, J. Cell Sci. 111 (Pt 13) (1998) 1853–1865.
[21] K. Omori, T. Kida, M. Hori, H. Ozaki, T. Murata, Multiple roles of prostaglandin E -EP signal in vascular permeability, Br. J. Pharmacol. 171 (2014) 4879–4889.
[22] F.R. Curry, R.H. Adamson, Vascular permeability modulation at the cell, microvessel, or whole organ level: towards closing gaps in our knowledge, Cardiovasc. Res. 87 (2010) 218–229.
[23] D.D. Ku, J.K. Zaleski, S. Liu, T.A. Brock, Vascular endothelial growth factor induces EDRF-dependent relaxation in coronary arteries, Am. J. Physiol. 265 (1993) H586–H592.
[24] B. Li, A.K. Ogasawara, R. Yang, W. Wei, G.W. He, T.F. Zioncheck, S. Bunting, A.M. de Vos, H. Jin, KDR (VEGF receptor 2) is the major mediator for the hypotensive effect of VEGF, Hypertension 39 (2002) 1095–1100.
[25] W. Wei, H. Jin, Z.W. Chen, T.F. Zioncheck, A.P. Yim, G.W. He, Vascular endothelial growth factor-induced nitric oxide- and PGI2-dependent relaxation in human internal mammary arteries: a comparative study with KDR and Flt-1 selective mutants, J. Cardiovasc. Pharmacol. 44 (2004) 615–621.
[26] H. Ashrafpour, N. Huang, P.C. Neligan, C.R. Forrest, P.D. Addison, M.A. Moses, R.H. Levine, C.Y. Pang, Vasodilator effect and mechanism of action of vascular endothelial growth factor in skin vasculature, Am. J. Physiol. Heart Circ. Physiol. 286 (2004) H946–H954.
[27] A. Abramsson, O. Berlin, H. Papayan, D. Paulin, M. Shani, C. Betsholtz, Analysis of mural cell recruitment to tumor vessels, Circulation 105 (2002) 112–117.