**Endothelial PAS Domain Protein 1 Gene Promotes Angiogenesis Through the Transactivation of Both Vascular Endothelial Growth Factor and Its Receptor, Flt-1**

Norihiko Takeda,* Koji Maemura,* Yasushi Imai, Tomohiro Harada, Daiji Kawanami, Takefumi Nojiri, Ichiro Manabe, Ryozo Nagai

**Abstract**—Endothelial PAS domain protein 1 (EPAS1) is a basic-helix–loop–helix/PAS domain transcription factor that is expressed preferentially in vascular endothelial cells. EPAS1 shares high homology with hypoxia-inducible factor-1α (HIF-1α) and is reported to transactivate vascular endothelial growth factor (VEGF), fetal liver kinase-1 (Flt-1), and Tie2 promoters. In this study, we analyzed the role of EPAS1 in the process of angiogenesis. Using microarray technology, we looked for target genes regulated by EPAS1 in vascular endothelial cells. A total of 130 genes were upregulated by EPAS1, including fms-like tyrosine kinase-1 (Flt-1). Reporter analysis using human Flt-1 promoter and gel mobility shift assays showed that the heterodimer of EPAS1 and aryl hydrocarbon receptor nuclear translocator binds directly to HIF-1α-binding site upstream of Flt-1 promoter and transactivates it. Small interfering RNA targeted to EPAS1 but not HIF-1α attenuated desferrioxamine-induced Flt-1 mRNA expression, thus EPAS1 is thought to play an essential role in hypoxic induction of Flt-1 gene. Furthermore, using mouse wound healing models, we demonstrated that adenovirus-mediated delivery of EPAS1 gene significantly induced the expression of VEGF, Flt-1, Flk-1, and Tie2 mRNA at the wound site and promoted mature angiogenesis. The proportion of the number of mural cells in newly formed vessels was significantly higher in EPAS1-treated wound area than VEGF-treated area. In conclusion, EPAS1 promotes Flt-1 gene expression and induces mRNA expression of VEGF, Flk-1, and Tie2, leading to enhancement of mature angiogenesis in vivo. Thus, EPAS1 may contribute to the construction of mature vessels by modulating the coordinated expressions of VEGF, Flt-1, Flk-1, and Tie2. 

**Key Words:** angiogenesis ■ EPAS1 ■ hypoxia ■ Flt-1

**H**ypoxia plays an extremely important role in the pathogenesis of many cardiovascular diseases. In hypoxic conditions, each organ or cell shows compensatory response at the molecular and cellular levels. Hypoxia-inducible factor-1α (HIF-1α) is expressed ubiquitously and serves as a master regulatory gene in the process of hypoxic response.1 Especially in the cardiovascular system, each organ promotes angiogenesis to increase oxygen and nutrient delivery. Endothelial PAS domain protein 1 (EPAS1) is a member of basic-helix–loop–helix/PAS domain containing transcription factor family and has a high homology to HIF-1α, thus also termed HIF-2α.2,3 Like HIF-1α, the EPAS1 protein is stabilized during hypoxia and forms a heterodimer with aryl hydrocarbon receptor nuclear translocator (ARNT) and transactivates the vascular endothelial growth factor (VEGF) promoter.4,5 Several groups generated EPAS1 knockout mice and reported that this resulted in the generation of different phenotypes. These results demonstrated that EPAS1 plays an essential role in the process of vascular remodeling during embryonic development6 as well as in catecholamine homeostasis7 and fetal lung maturation.8 Thus, HIF-1α and EPAS1 are thought to play critical roles during the angiogenic hypoxia response.

In contrast to HIF-1α, EPAS1 is expressed mainly in vascular endothelial cells. Therefore, EPAS1 is thought to function differently from HIF-1α. Indeed, EPAS1 was reported to transactivate endothelial-specific genes such as Tie2 and fetal liver kinase-1 (Flk-1).2,9,10 However, the precise profiles of genes induced by EPAS1 and the functions of EPAS1 in vascular endothelial cells have not been elucidated.

VEGF is the most important angiogenic factor, and its function is essential in embryonic vasculogenesis as well as angiogenesis in the adult.11,12 However, VEGF alone is not sufficient to assemble physiologically functional vasculature. Results obtained from therapeutic angiogenesis studies using VEGF showed that new vessels induced by VEGF are leaky13 and do not persist for long.14 Thus, in addition to VEGF,
some other molecules are expected to contribute to production of functional and lasting mature vessels. To elucidate angiogenesis molecular mechanisms in terms of vessel maturation, it may be beneficial to clarify the target genes of EPAS1 besides VEGF.

In this study, we used microarray technology to identify the genes that were upregulated by EPAS1 in vascular endothelial cells. A total of 130 genes were upregulated by EPAS1, including VEGF receptor 1 (VEGF-R1 or fms-like tyrosine kinase-1 [Flt-1]), implying that it is regulated by EPAS1. We demonstrate that the heterodimer of EPAS1 and ARNT binds to the HIF-1–binding site upstream of Flt-1 promoter and transactivates the promoter activity. Moreover, using small interfering RNA (siRNA) against EPAS1 or HIF-1α, we found that EPAS1 is essential in hypoxic regulation of Flt-1 mRNA expression. Furthermore, EPAS1 gene delivery to wound tissue enhanced the expression of VEGF, Flt-1, Flk-1, and Tie2 in vivo, resulting in promotion of angiogenesis and acceleration of the wound healing process. We show that EPAS1 enhances not only VEGF expression but also the concomitant expression of its 2 receptors, Flk-1 and Flt-1, as well as Tie2. EPAS1 may promote angiogenesis and contribute to mature vessel establishment by activating VEGF gene expression and also affecting coexpression of related endothelial-specific receptors involved in the process.

Materials and Methods

Cell Culture

Human umbilical vein endothelial cells (HUVECs) were obtained from BioWhittaker (Walkersville, Md) and were maintained in endothelial growth medium 2 (BioWhittaker). Bovine aortic endothelial cells (BAECs) were primary cells cultured in DMEM (Sigma) containing 10% FBS. To mimic hypoxic conditions, cells were treated with 130 μmol/L desferrioxamine (Sigma) for 16 hours.

Construction of Plasmids and Recombinant Adenovirus

The pGL2-Basic plasmid was purchased from Promega. To generate the Flt-1 reporter plasmid (pGL2Flt1), the promoter region of the Flt-1 gene from position −1160 to +305 was amplified by polymerase chain reaction (PCR) and subcloned into pGL2-Basic plasmid. Successive deletion of the 5′-flanking region was performed by PCR. PCR products were inserted between SacI and BglII restriction sites of pGL2-Basic promoter to yield pGL2Flt1 (−886 to +305) and pGL2Flt1 (−334 to +305). A variation of the pGL2Flt1 plasmid pGL2Flt1mut was constructed; this was identical to pGL2Flt1 except that the HIF-1–binding site (bp −965 to −958) was mutated from AACGTGGA to AAAAAAGGA. The EPAS1 expression vector pHIP-1 was a gift from Steven L. McKnight (University of Texas Southwestern Medical Center, Dallas). The plasmid pARNT was constructed as described previously. For mouse VEGF164 cDNA isolation, PCR was performed using total RNA from mouse heart. The primers used were 5′-ATGAACTTTCCTGGTTTGTGG and 5′-ACCCGCGCTGGTTTGTGTATC. Recombinant adenoviruses were prepared using Ad-Easy system as described previously, and those expressing β-galactosidase (βGAL), EPAS1 (1-870), or mouse VEGF164 were designated AdCMV βGAL, AdCMV.EPAS1(1-870), or AdCMV.mVEGF164, respectively.

Microarray Analysis

Microarray analysis was performed using ~12 000 human cDNA (Agilent Technologies; Palo Alto, Calif) as described previously. In the first instance, HUVECs were infected with AdCMV.EPAS1-

### Table: Representative Genes Upregulated by EPAS1

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(1-870) or AdCMV βGAL. Forty-eight hours after infection, total RNA was isolated from cell cultures and applied to microarray analysis. A threshold of a 2-fold change in expression was used for comparison of AdCMV.EPAS1(1-870) and AdCMV βGAL-infected HUVECs.

Northern Blot Analysis

Total RNA was isolated from HUVECs using RNasey method (Qiagen). Northern blot analysis was performed as described. To correct for differences in RNA loading, the membranes were rehybridized with a radiolabeled 18S oligonucleotide.

Western Blot Analysis of EPAS1 Protein Expression

Whole-cell protein extracts or nuclear extracts were prepared from HUVECs. A total of 20 μg of protein per sample was evaluated by Western blot analysis performed as described. EPAS1 was detected using polyclonal anti-hHIF-2α antibody (Novus; 1:1000) and as a secondary antibody, anti-rabbit horseradish peroxidase–conjugated IgG (Cell Signaling; 1:2000) followed by enhanced chemiluminescence plus detection (Amersham).

Figure 1. Overexpression of EPAS1 induces Flt-1 mRNA. HUVECs were infected with AdCMV.EPAS1(1-870) or control, AdCMV βGAL, at the indicated moi (MOI) for 2 hours at 37°C. Total RNA was isolated 48 hours after infection. Northern blot analysis was performed using human Flt-1 cDNA as a probe. The same blot was rehybridized with a cDNA probe for human EPAS1 to confirm the expression of adenovirus. Induction of EPAS1 protein expression was also confirmed by Western blot analysis using whole-cell extracts.
Transient Transfection Assay

BAECs were cultured in 12-well plates, and transient transfection was performed with 0.6 μg of total DNA using Lipofectamine reagent (Invitrogen) as described previously. 18 To correct for variation in transfection efficiency, we cotransfected 0.1 μg of pCMV-βgal in all experiments. Data for each construct are presented as the mean±SEM.

In Vitro Transcription and Translation/Gel Mobility Shift Assay

In vitro transcription and translation of EPAS1 and ARNT and gel mobility shift assays were performed as described. 18 The sequence of the double-stranded oligonucleotide containing the HIF-1α binding site (5'-CATATTGAGAACACTGGAGATTATGTCAATCG-3') was derived from the sequence of the Flt-1 promoter corresponding to positions −979 to −945. For supershift analysis, 1 μg of anti-myc antibody (Invitrogen) was preincubated with in vitro-translated proteins for 1 hour at room temperature before binding reaction.

siRNA Duplexes and Transfection

The siRNA oligonucleotides were designed after recommendations of Elbashir et al 19 and were synthesized using Silencer siRNA Construction Kit (Ambion). The EPAS1 siRNA duplex targeted nucleotides 830 to 848 of EPAS1 mRNA sequence (U81984) and comprised sense 5'-ACCAACAGCATCAUCAGdTdT-3' and antisense 5'-GGAUGGGCUGUGAUUGUTdT-3'. The HIF-1α siRNA duplex targeted nucleotide 1521 to 1539 of HIF-1α mRNA sequence (NM001530) and comprised sense 5'-CUGAUGACCGA-CAACUUGAdTdT-3' and antisense 5'-UCAAAGUGCGGU-CAUCAGdTdT-3' as described previously. 20 The scramble EPAS1 control duplex did not target any gene and comprised sense 5'-UAGGUGAGAGAGUGGCGdTdT-3' and antisense 5'-GCGCCACUCUCUCUACCUAdTdT-3'. Duplexes were prepared according to the instructions of the manufacturer. HUVECs were plated onto 10-cm cell culture dishes and grown to 70% confluence according to the instructions of the manufacturer. HUVECs were harvested with 0.05% trypsin and were washed with PBS. Cell migration assays were performed with the Transwell (Corning Costar Japan) system as described. 21,22 Briefly, HUVECs were infected with adenovirus and added to the upper chamber of the Transwell. The lower chamber was filled with serum-free Eagle basal medium containing 50 ng/ml of VEGF. After 5 hours, the cells present beneath the membrane were fixed with methanol and stained with Giemsa solution. 22 Cells were counted in 3 random fields per well under low magnification using a microscope. Analysis was performed on 3 wells for each condition, and each experiment was repeated 3×.

Wounding, Adenovirus Infection, and Wound Tissue Preparation

All animal procedures in these studies were designed in accordance with the Guide for Animal Experimentation (Faculty of Medicine, University of Tokyo). Five-week-old male BALB/c mice (Charles River Japan; Yokohama, Japan) were used in the experiment (n=5). Mice were anesthetized by intraperitoneal administration of 0.15 mL of 3.6% chloral hydrate. Four full-thickness wounds were made on the back of mice. 23 The wounds were infected with recombinant adenovirus (5×10⁹ plaque-forming units) expressing βgal, human EPAS1(-1-870), or mouse VEGF164. Infection was performed by 4 direct subcutaneous injections around the wound using a 27-gauge needle. The progress of wound closure was analyzed daily as described. 24 Healing was expressed as the percent decrease in wound area relative to the original wound area. Six days after wounding, the mice were euthanized with carbon dioxide inhalation, and the wound tissues were collected.

Quantitative Real-Time RT-PCR

Quantitative RT-PCR was performed as described previously. 25 Primers and probes were obtained as assay on demand and used according to the protocol of the manufacturer (Applied Biosystems; Foster City, Calif). The level of transcripts for the constitutive housekeeping gene product GAPDH was measured quantitatively in each sample to control for sample-to-sample differences in RNA concentration.

Immunohistochemistry and Analysis of Capillary Density

Immunohistochemical analysis of mouse tissue was performed as described previously. 26 To identify endothelial cells, we used rat monoclonal antibody directed against mouse CD31 (BD Biosciences; Palo Alto, Calif). In addition, mouse monoclonal antibody against smooth muscle (SM) α-actin (Sigma) was used to identify mural cells. Capillary densities, identified by positive staining for CD31 and appropriate morphology, were counted by a single observer blinded to the treatment regimen (mean number of capillaries per low-power field).

Figure 2. Heterodimer of EPAS1 and ARNT transactivates the Flt-1 promoter through its HIF-1α-binding site. A, EPAS1 and ARNT transactivate the Flt-1 promoter. B, Deletion analysis of Flt-1 promoter shows the promoter region between −1160 and −866 is responsible for transactivation by EPAS1 and ARNT. C, Mutation of the HIF-1α-binding site of the Flt-1 promoter abolished the transactivation. phEP-1 (EPAS1) or phARNT (ARNT; 0.6 μg) and pGL2hFlt-1 (0.6 μg) were cotransfected into BAECs. For all constructs (A–C), pCMV, βgal (0.1 μg) was cotransfected to correct for differences in transfection efficiency. The ratio of luciferase activity to βgal activity in each sample served as a measure of normalized luciferase activity. Fold induction represents the ratio (mean±SE) of normalized luciferase activity in cells transfected with expression plasmid to that in cells transfected with empty vector (pcDNA3). Each transfection experiment was performed at least 4× in triplicate. Data for each construct are presented as means±SE (P<0.05).
EPAS1 Transactivates the Flt-1 Promoter

To further elucidate the mechanism by which EPAS1 increases the Flt-1 mRNA levels, we first generated a reporter plasmid pGL2hFlt-1 containing a 1.6-kb fragment of the human Flt-1 promoter placed upstream of the luciferase reporter gene. This reporter plasmid construct was then cotransfected into BAECs together with the human EPAS1 expression plasmid pEP-1 and the human ARNT expression plasmid pARNT. EPAS1 and ARNT increased Flt-1 promoter activity by 2.5-fold (Figure 2A). Deletion of bp −1160 to −886 of the Flt-1 promoter markedly diminished this induction by EPAS1 and ARNT (Figure 2B). We examined this EPAS1-responsive element of the Flt-1 promoter from bp −1160 to −886 in detail and found an HIF-1–binding consensus sequence in −965 region. To determine whether EPAS1 transactivates the Flt-1 promoter by binding to this HIF-1–binding site, we made a mutant Flt-1 promoter with a 3-bp mutation at −969 to −965 (ACGTG to AAAAG). A reporter plasmid harboring this mutated site (pGL2hFlt-1mut) was again transfected into BAECs in the presence or absence of EPAS1 and ARNT plasmid constructs. Mutation of the HIF-1–binding site abolished the transactivation of Flt-1 promoter by EPAS1 (Figure 2C).

**Figure 3.** EPAS1 and ARNT directly bind to the HIF-1–binding site within the Flt-1 promoter. Direct binding of EPAS1/ARNT heterodimer to the HIF-1–binding site of Flt-1 promoter was confirmed (*). A, Gel mobility shift assays were performed using the indicated in vitro–translated proteins and a 35-bp double-stranded oligonucleotide (5′ CATAATTGAGGAAACAGTGGAATAGTGTCATCG 3′) probe containing the HIF-1–binding site derived from sequence of the Flt-1 promoter. Binding experiments were also performed in the presence of unlabeled HIF-1–binding consensus oligonucleotide (S) or unrelated nonspecific oligonucleotide (NS) at 10- or 100-fold molar excess. Addition of anti-myc antibody (Ab) interfered with binding. B, Gel mobility shift assays using a probe containing a 3-bp mutation at HIF-1 site (5′ CATAATTGAGGAAACAAAGGAATTAGTGTCATCG 3′).

**Results**

Microarray Analysis

To identify the downstream target genes of EPAS1, we first generated adenoviral constructs AdCMV.EPAS1-(1-870) and AdCMV.βGAL. We infected AdCMV.EPAS1-(1-870) or AdCMV.βGAL into HUVECs and isolated RNA after 48 hours. Differential hybridization was performed on microarray slides containing labeled RNA from AdCMV.

EPAS1–ARNT Heterodimer Binds to the HIF-1–Binding Site of Flt-1 Promoter

To examine whether the heterodimer of EPAS1 and ARNT binds directly to this HIF-1–binding site, we performed gel mobility shift assays with in vitro–translated EPAS1 and ARNT proteins and a labeled probe containing the HIF-1–binding site. In the presence of EPAS1 and ARNT proteins, DNA-binding activity was detected (Figure 3A); however, 3-bp mutation at HIF-1 site of this probe abolished protein binding (Figure 3B). The binding was attenuated with unla-
beled specific competitor containing HIF-1α-binding site but not with nonspecific competitor containing the specificity protein 1 element. The in vitro translated EPAS1 protein was myc tagged. Thus, we performed supershift analysis using anti-myc antibody. Addition of 1 μg/mL of anti-myc antibody in this reaction mixture interfered the binding (Figure 3A).

Knockdown of EPAS1 but not HIF-1α Attenuates Desferrioxamine-Induced Flt-1 mRNA Expression
To elucidate whether EPAS1 or HIF-1α is involved in hypoxic induction of Flt-1 gene expression, we generated siRNA against EPAS1 and HIF-1α. Desferrioxamine, which chemically mimics hypoxic conditions, markedly increased Flt-1 mRNA abundance in HUVECs transfected by scramble siRNA as a control. Knockdown of HIF-1α by siRNA did not affect Flt-1 mRNA induction by desferrioxamine. On the other hand, knockdown of EPAS1 markedly attenuated Flt-1 gene expression enhancement (Figure 4A and 4B).

EPAS1 Enhances Chemotactic Response Toward VEGF
To evaluate the role of EPAS1 on the migration activity of vascular endothelial cells, Boyden chamber analysis was performed using HUVECs. When HUVECs were infected with AdCMV.EPAS1-(1-870); 10 multiplicities of infection [mois]), their chemotactic activities toward VEGF were significantly increased compared with those treated with AdCMV.βGAL (Figure 5).

Figure 4. Knockdown of EPAS1 attenuates desferrioxamine (DFX)-induced Flt-1 expression. A, Transfection of siRNA against HIF-1α did not affect Flt-1 mRNA induction by DFX. However, siRNA against EPAS1 markedly attenuated Flt-1 gene expression enhancement. Scramble siRNA was used as a control. The same blot was rehybridized with an oligonucleotide probe for 18S rRNA to display differences in loading. Inhibitory effect of siRNA on responsiveness of EPAS1 protein to DFX was confirmed by Western blot analysis using nuclear extracts. B, Densitometric analysis of Flt-1 mRNA expression was shown as mean±SE. (*P<0.05 compared with DFX-nontreated cells).

Figure 5. EPAS1 potentiates vascular endothelial cell chemotaxis toward VEGF. HUVECs were infected with adenovirus at 10 moi for 1 day in the presence of serum and then serum starved for 5 hours before chemotaxis assay was performed. Quantitative data of Giemsa-stained HUVECs that migrated through filters toward VEGF (50 ng/mL) are shown. (*P<0.01 comparing βGAL with EPAS1; n=6).

EPAS1 Gene Delivery Promotes Angiogenesis at Wound Site
To examine whether EPAS1 promotes angiogenesis in vivo, we analyzed the effect of EPAS1 overexpression in the healing process of skin wound, which is commonly used as a model of angiogenesis. First, full-thickness wounds were created in the back skin of mice and then the wound site was treated with AdCMV.EPAS1-(1-870), AdCMV.βGAL, or saline as a control. In mice treated with AdCMV.EPAS1-(1-870), the healing process was significantly accelerated compared with mice treated with AdCMV.βGAL or saline (Figure 6A). In mice treated with AdCMV.EPAS1-(1-870), the wounds closed 2 to 3 days faster relative to AdCMV.βGAL- or saline-treated mice (Figure 6B). Tissue sections from the wound sites were examined histologically. There were significantly increased numbers of newly formed platelet-endothelial cell adhesion molecule-1 (PECAM-1)-positive vessels (Figure 6C) and increased capillary density (Figure 6D) in AdCMV.EPAS1-(1-870)-treated mice 7 days after the injury, compared with AdCMV.βGAL or saline (P<0.05). Moreover, the expression of VEGF, Flt-1, Flk-1, and Tie2 were all upregulated at the wound site in mice treated with AdCMV.EPAS1-(1-870) compared with controls (Figure 6E).

EPAS1 Gene Delivery Enhances Formation of New Vessels With SM α-Actin–Positive Mural Cells
To compare the effect of EPAS1 and VEGF in angiogenesis, we generated adenovirus-expressing mouse VEGF164 (AdCMV.mVEGF164). Delivery of mVEGF164 gene markedly enhanced capillary density at the wound site. However, newly formed vessels induced by mVEGF164 have fewer SM α-actin–positive mural cells compared with those induced by EPAS1 (Figure 7).
In this study, we examined the role of EPAS1 in vascular endothelial cells by analyzing the downstream genes of EPAS1 using microarray technique. A total of 130 genes were upregulated, notably including that of Flt-1. We also showed that the Flt-1 promoter is activated by EPAS1, and the induction is dependent on its HIF-1α-binding site. In addition, EPAS1 enhanced the migratory response of HUVECs induced by VEGF. Furthermore, EPAS1 gene delivery promoted the angiogenic process in vivo by enhancing the concomitant expression of VEGF, Flt-1, Flk-1, and Tie2. EPAS1 has high homology to HIF-1α/HIF-1β, and their functions are increased during hypoxia. Furthermore, both transactivate the VEGF promoter. However, in contrast to HIF-1α, EPAS1 is expressed mainly in vascular endothelial cells and transactivates promoters of endothelial-specific genes Flk-1 and Tie2.

**Discussion**

In this study, we examined the role of EPAS1 in vascular endothelial cells by analyzing the downstream genes of EPAS1 using microarray technique. A total of 130 genes were upregulated, notably including that of Flt-1. We also showed that the Flt-1 promoter is activated by EPAS1, and the induction is dependent on its HIF-1α-binding site. In addition, EPAS1 enhanced the migratory response of HUVECs induced by VEGF. Furthermore, EPAS1 gene delivery promoted the angiogenic process in vivo by enhancing the concomitant expression of VEGF, Flt-1, Flk-1, and Tie2.

EPAS1 has high homology to HIF-1α, and their functions are increased during hypoxia. Furthermore, both transactivate the VEGF promoter. However, in contrast to HIF-1α, EPAS1 is expressed mainly in vascular endothelial cells and transactivates promoters of endothelial-specific genes Flk-1 and Tie2.
EPAS1 and Flt-1 have very similar characteristics; that is, they are expressed mainly in vascular endothelial cells, and their expression is upregulated during hypoxia. On the basis of microarray analysis results, we hypothesized that EPAS1 directly upregulated the expression of Flt-1. Flt-1 promoter activity is known to be activated during hypoxia, and the hypoxia-responsive element within the Flt-1 promoter has also been identified. However, the precise molecular mechanisms of transactivation of Flt-1 remained unclear. Moreover, it has not been elucidated whether HIF-1α, EPAS1, or both are involved in hypoxic induction of Flt-1 gene expression. In this study, we demonstrated that the heterodimer of EPAS1 and ARNT binds directly to the HIF-1 – binding sequences ACGTG within the hypoxia-responsive element and transactivates the Flt-1 promoter. In addition, we discovered that EPAS1 but not HIF-1α is important in hypoxic induction of Flt-1 gene expression by using siRNA for the first time. Therefore, these results are of utmost importance on the basis of our assertion that almost no gene had been identified as an EPAS1-specific target gene so far. Moreover, we have shown the novel means to discriminate the roles of EPAS1 and HIF-1α. Collectively, the results of this study suggest that EPAS1 upregulates not only VEGF but also endothelial-specific receptors Flt-1, Flk-1, and Tie2, all of which are essential in the angiogenesis process.

In this study, delivery of EPAS1 gene to wound tissue upregulates the expressions of VEGF, Flt-1, Flk-1, and Tie2 at the wound site, which results in promotion of mature angiogenesis. The roles of Flk-1 and VEGF in angiogenesis have been well established. In contrast, in angiogenesis, the functional role played by Flt-1 is still unclear. For instance, Flt-1 was reported to negatively regulate angiogenesis through sequestering VEGF and diminishing the Flk-1– mediated angiogenic stimulus. However, recent reports have shown that at least in pathological conditions such as tumor angiogenesis, inhibition of Flt-1 by antagonists impairs pathological angiogenesis; thus, in such situations, Flt-1 indeed promotes angiogenesis. More recently, Flt-1 stimulation by placental growth factor (PIGF) was reported to transphosphorylate Flk-1 and amplify Flk-1 activation by VEGF. Moreover, heterodimers of Flk-1/Flt-1 and VEGF/PIGF were reported to exist and promote angiogenesis. Thus, the coordinated function of Flt-1 and Flk-1 is likely to be important in the angiogenesis process.

For therapeutic angiogenesis, there are clinical studies under way using VEGF gene or protein. VEGF overexpression indeed promotes angiogenesis; however, the newly formed vessels induced by VEGF were leaky and tended to regress soon after reduction of VEGF concentration. In this study, most vessels induced by VEGF gene lack SM α-actin–positive mural cells, therefore, they should be considered more immature vessels. Thus, the angiogenic process modulated by EPAS1 appears to be similar to that observed in physiological angiogenesis during hypoxic conditions. The spatiotemporal expressions of some ligands and receptors have been reported to be involved in assembling mature vessels. Particularly, angiopeotin-1 stabilizes the vessel through Tie2 receptors, and in combination with VEGF, it contributes to form mature vessels. PIGF also promotes angiogenesis through Flt-1–mediated signals. In this model, EPAS1 delivery upregulated both Tie2 and Flt-1 gene expression. Thus, EPAS1 may promote well-organized vascular formation by coordinately regulating expression not only of VEGF but also endothelial-specific receptors, those that play essential roles in the process.

In conclusion, EPAS1 coordinately enhanced expression of VEGF and endothelial-specific receptors Flt-1, Flk-1, and Tie2 and promoted mature angiogenesis. Therefore, studies on EPAS1 functions would provide us with further insights into the complex regulatory network of vessel maturation.

Acknowledgments

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References

action of hypoxia-inducible factor-2alpha (HIF-2alpha) and Ets-1 in the transcriptional activation of vascular endothelial growth factor receptor-2 (Flk-1). J Biol Chem. 2003;278:7520–7530.


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