Generation of a Dominant-negative Mutant of Endothelial PAS Domain Protein 1 by Deletion of a Potent C-terminal Transactivation Domain*

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Endothelial PAS domain protein 1 (EPAS1) is a basic helix-loop-helix/PAS domain transcription factor that is preferentially expressed in vascular endothelial cells. EPAS1 shares high homology with hypoxia-inducible factor-1α (HIF-1α) and, like HIF-1α, has been shown to bind to the HIF-1-binding site and to activate its downstream genes such as vascular endothelial growth factor (VEGF) and erythropoietin. In this report, we show that EPAS1 increased VEGF gene expression through the HIF-1-binding site. This transactivation was enhanced further by cotransfection of an aryl hydrocarbon receptor nuclear translocator expression plasmid. Deletion analysis of EPAS1 revealed a potent activation domain (amino acids 486–639) essential for EPAS1 to transactivate the VEGF promoter. We confirmed the ability of this domain to activate transcription using a Gal4 fusion protein system. Because a truncated EPAS1 protein lacking the transactivation domain at amino acids 486–639 eliminated induction of the VEGF promoter by wild-type EPAS1, the truncated protein functions as a dominant-negative mutant. Most important, infection of the cells with an adenoviral construct expressing this mutant inhibited the induction of VEGF mRNA under conditions that mimic hypoxia. Our results suggest that EPAS1 is an important regulator of VEGF gene expression. Since VEGF plays a crucial role in angiogenesis, the ability of dominant-negative EPAS1 to inhibit VEGF promoter activity raises the possibility of a novel approach to inhibiting pathological angiogenesis.

Endothelial PAS domain protein 1 (EPAS1/HIF/HIF/MOP2) is a member of the transcription factor family characterized by a basic helix-loop-helix domain and a PAS domain composed of two imperfect repeats (1–4). The acronym PAS derives from the first three members of the family: the period gene (5, 6), the aryl hydrocarbon receptor nuclear translocator (ARNT) gene (7), and the single-minded gene (8). Using helix-loop-helix and PAS domains, proteins of this family form heterodimers that bind to target genes through the basic region and govern important biological functions. For example, period and clock are important in the regulation of circadian rhythm (5, 6, 9); the aryl hydrocarbon receptor is involved in xenobiotic metabolism (10); and tracheless determines the fate of tracheal cells (11).

Among proteins of the PAS domain family, EPAS1 shares the greatest amino acid homology (48% overall identity) with hypoxia-inducible factor-1α (HIF-1α) (1–4, 12). HIF-1α plays a crucial role in cellular adaptation to hypoxia by inducing genes for erythropoietin, vascular endothelial growth factor (VEGF), and the glycoplycemic enzymes (12–15). The basic helix-loop-helix and PAS domains in EPAS1 and HIF-1α share a particularly high degree of homology (83% identity in the basic helix-loop-helix domain and 66% identity in the PAS domain). Like HIF-1α, EPAS1 heterodimerizes with ARNT and binds to the HIF-1-binding site. However, the pattern of EPAS1 expression is distinct from that of HIF-1α. EPAS1 is expressed preferentially and at high levels in vascular endothelial cells from early development to adulthood. HIF-1α, in contrast, is expressed ubiquitously, but at low levels (1, 2, 4, 16, 17). Flame et al. (18) reported recently that VEGF expression is up-regulated markedly in hemangioblastomas and that this up-regulation correlates highly with elevated EPAS1 expression, but not HIF-1α expression. These data suggest that the role EPAS1 plays in pathological angiogenesis is distinct from the role HIF-1α plays. The functional properties and domain structure of EPAS1, however, had not been characterized until this report.

We show in vascular endothelial cells that EPAS1 transactivates the VEGF promoter through its HIF-1-binding site. Using a series of deletion mutants, we have identified a potent activation domain within the EPAS1 C terminus. By gene fusion experiments, we show that this activation domain is sufficient to confer transactivity to a Gal4 DNA-binding domain. We also show that a truncated EPAS1 protein that lacks the transactivation domain functions as a dominant-negative mutant and inhibits VEGF gene induction by desferrioxamine.

Experimental Procedures

Cell Culture—Human umbilical vein endothelial cells (HUVECs) were obtained from Clonetics Corp. (San Diego, CA) and cultured in M199 medium supplemented with 20% fetal calf serum (HyClone Laboratories, Logan, UT), 60 μg/ml endothelial cell growth supplement (Collaborative Biomedical, Bedford, MA), and 50 mg/ml heparin as...
described (19). Bovine aortic endothelial cells (BAECs) were isolated from calf aortas and cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum as described (20, 21). Cells were passaged every 2–3 days, and cells from passages 5–7 were used for the experiments.

Construction of Plasmids—The pG2L-Basic plasmid containing the firely luciferase reporter gene was purchased from Promega (Madison, WI). The pGL2hVEGF reporter plasmid was constructed by inserting the human VEGF promoter from base pairs (bp) −2362 to +61 into pG2L-Basic (22, 23). pGL2hVEGFmut was identical to pGL2hVEGF, except that the HIF-1-binding site (bp −975 to −968) was mutated from TACGTCTGGG to TCTCGAGG. The pGL2hVEGF expression vector pEPI-1, which contains the entire coding sequence of human EPAS1 in the eukaryotic expression vector pCDNA3 (Invitrogen, Carlsbad, CA), was a gift from Steven L. McKnight (University of Texas Southwestern Medical Center, Dallas, TX) (4). In the C-terminal deletion experiments described in this study, we designated this plasmid as pEPI-1-(1–870).

To construct pEPI-1-(1–X) C-terminal deletion mutants, we amplified a section of pEPI-1 encoding amino acids 279 to X of the EPAS1 cDNA by polymerase chain reaction (PCR) with Pfu DNA polymerase (Stratagene, La Jolla, CA). The specific 5′-primers contained an EcoRI site, and the 3′-primers contained an XbaI site. The PCR products were digested with EcoRI and XbaI, and the fragment was used to replace the corresponding site in pEPI-1.

To generate the pHIF-1α, we first amplified a 2622-bp cDNA fragment containing the entire open reading frame of human HIF-1α by reverse transcriptase-PCR with human leukocyte total RNA and Pfu DNA polymerase. The PCR fragment was then cloned into pCDNA3. phARN1 was subcloning a BamHI fragment containing full-length ARNT-coding sequences from pBM5/NEO-M1-1 (a gift from Oliver Hankinson, University of California, Los Angeles, CA) (7) into pCDNA3. For the Gal4-hEPAS1 fusion constructs, hEPAS1 fragments were fused in frame to the C terminus of the Gal4 DNA-binding domain (amino acids 1–147) of pSG424 as described (24). The reporter pGLuc (containing five Gal4-binding sites upstream of the adenovirus E1b gene) and the luciferase reporter gene were obtained from Promega. The authenticity of all constructs was verified by dyeoxy chain termination sequencing.

Construction of Recombinant Adenoviruses—Recombinant adenoviruses were prepared with standard homologous recombination techniques using the replication-defective E1/E3-deleted serotype 5 human adenovirus (25). A Muc tag sequence was added to the 3′-end of the cDNA fragment of EPAS1 (1–870) or EPAS1-(1–485). We then used these fragments to replace the β-galactosidase fragment of the pAdEasy-1 adenoviral shuttle plasmid, in which the expression of β-galactosidase cDNA is under the control of the cytomegalovirus (CMV) promoter/enhancer (25). These shuttle plasmids were cotransfected with the plasmid pJM17 into 293 cells. Recombinant adenovirus clones were isolated from a single plaque, expanded in 293 cells, and purified by cesium chloride ultracentrifugation. The titer of the purified adenovirus was determined in 293 cells by plaque assay techniques. Recombinant adenoviruses expressing β-galactosidase, EPAS1-(1–870), or EPAS1-(1–485) were designated as AdCMV.βGal, AdCMV.EPAS1-(1–870), and AdCMV.EPAS1-(1–485), respectively. Expression of AdCMV.EPAS1-(1–870) and AdCMV.EPAS1-(1–485) was confirmed by Western blotting using an anti-c-Myo antigen antibody (9E10, Calbiochem-Novabiochem, San Diego, CA) and cell extracts from the infected HUVECs.

RNA Isolation and Northern Analysis—Total RNA was isolated from HUVECs by guanidinium isothiocyanate extraction and centrifugation through cesium chloride according to standard protocols (21). Total RNA (10 μg) was fractionated on a 1.3% formaldehyde-agarose gel and transferred to Nitroblot filters (MSI, Westborough, MA). The filters were then hybridized with 32P-labeled, randomly primed cDNA probes for 1 h at 68 °C in Quick-hyb solution (Stratagene). The hybridized filters were washed in 30 mM NaCl, 3 mM sodium citrate, and 0.1% SDS at 55 °C and autoradiographed on Eastman Kodak XAR film at ~80 °C. A 1.0-kilobase BamHI-EcoRI fragment of hEPAS1 cDNA or the 538-bp (bp 58–595) human VEGF cDNA fragment amplified by reverse transcriptase-PCR according to the published human VEGF cDNA sequence was used as a probe (26). To correct for differences in RNA loading, the filters were rehybridized with a radio labeled 18S oligonucleotide which is expressed in the presence or absence of 1,25(OH)2 vitamin D3 methionine according to the manufacturer’s instructions.

Gel Mobility Shift Assay—The sequence of the double-stranded oligonucleotide containing the HIF-1-binding site (5′-CCACAGTGCAT-ACGTTGGGCTCCAAACGAGTCCTTCTT-3′) was derived from the sequence of the VEGF promoter (23). Gel mobility shift assays were performed as described (21). A typical binding reaction mixture contained 25,000 cpm probe, 5 μl of in vitro translated protein, 50 ng of poly(dI-dC)poly(dI-dC), 25 mM HEPS, pH 7.9, 40 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, and 10% glycerol. The reaction mixture was incubated at room temperature for 20 min and fractionated on 5% polyacrylamide gels in 0.25 × buffer containing 22 mM Tris base, 22 mM boric acid, and 0.5 mM EDTA.

Transient Transfection Assays—Cells were transfected with 0.15 μg of reporter construct and 0.5–1 μg of expression construct using LipofectAMINE (Life Technologies, Inc.) according to the manufacturer’s instructions. To correct for variation in transfection efficiency, we cotransfected 0.15 μg of pCAT3-Control (Promega) in all experiments. In some experiments, 24 h after transfection, cells were treated with 130 μg desferrioxamine (Sigma) for an additional 24 h. Cell extracts were prepared 48 h after transfection by a detergent lysis method (Promega). Luciferase activity and chloramphenicol acetyltransferase (CAT) activity were measured as described (21). The ratio of luciferase activity to CAT activity in each sample served as a measure of normalized luciferase activity. Each construct was transfected at least four times, and each transfection was done in triplicate. Data for each construct are presented as the means ± S.E.

Statistics—Comparisons between groups were made by a factorial analysis of variance followed by Fisher’s least significant difference test when appropriate. Statistical significance was accepted at p < 0.05.

RESULTS

Overexpression of EPAS1 Induces VEGF mRNA—Although EPAS1 has been shown to transactivate the VEGF promoter by transient transfection assays, it was not known whether EPAS1 increases the levels of VEGF mRNA (1). To test this hypothesis, we first generated an adenoviral construct (AdCMV.EPAS1-(1–870)) that contains a full-length EPAS1 cDNA. Infection with adenoviruses expressing β-galactosidase, EPAS1-(1–870), or EPAS1-(1–485) did not increase the VEGF mRNA levels (1). To confirm this hypothesis, we generated an adenoviral vector containing a human EPAS1 expression plasmid pEP-1 and the reporter plasmid pGL2hVEGF containing a 2.3-kilobase fragment of the human VEGF promoter upstream of the luciferase reporter gene. EPAS1 increased VEGF promoter activity in vitro (26).

In Vitro Transcription and Translation—The human EPAS1 cDNA was subcloned into the HIV-1 expression vector pEPI-1 and its C-terminal deletion mutants contained a T7 RNA polymerase promoter for in vitro expression. In vitro transcription and translation were carried out using the TNT-coupled reticulocyte lysate system (Promega) in the presence or absence of 1,25(OH)2 vitamin D3 methionine according to the manufacturer’s instructions.

FIG. 1. Overexpression of EPAS1 induces VEGF mRNA. HUVECs were infected with AdCMV.EPAS1-(1–870) or, as a control, AdCMV.βGal at the indicated multiplicity of infection (MOI) for 2 h at 37 °C. Total RNA was isolated at 48 h after infection. Northern blot analysis was performed using a human VEGF probe. The same blot was rehybridized with a human EPAS1 probe to confirm the expression of EPAS1 and an 18S oligonucleotide to display differences in loading.

Overexpression of EPAS1 Transactivates the VEGF Promoter through the HIF-1-Binding Site—To further elucidate the mechanism by which EPAS1 increases the VEGF mRNA levels, we cotransfected into BAECs the human EPAS1 expression plasmid pEP-1 and the reporter plasmid pGL2hVEGF containing a 2.3-kilobase fragment of the human VEGF promoter upstream of the luciferase reporter gene. EPAS1 increased VEGF promoter activity in vitro (26).
by 17-fold (Fig. 2A). This up-regulation of VEGF promoter activity by EPAS1 appears to be specific because cotransfection of the EPAS1 expression plasmid had no effect on endothelin-1 promoter or SV40 promoter activity (data not shown).

EPAS1 is known to bind to the HIF-1-binding site (4). To determine whether EPAS1 transactivates the native VEGF promoter by binding to an HIF-1-binding site, we mutated the HIF-1-binding site at bp 2975 to 2968 (TACGTGGG to TCTC-GAGG) of the VEGF promoter. A reporter plasmid harboring this mutated site (pGL2hVEGFmut) was transfected into BAECs in the presence and absence of EPAS1. Mutation of the HIF-1-binding site dramatically reduced induction of the VEGF promoter by EPAS1 (Fig. 2A). These data indicate that EPAS1 transactivates the VEGF promoter mainly through its HIF-1-binding site.

EPAS1 has been shown to heterodimerize with ARNT (4). To determine whether heterodimerization between EPAS1 and ARNT is important for transactivation of the VEGF promoter, we performed cotransfection experiments. EPAS1 alone increased VEGF promoter activity by 15-fold (Fig. 2B), whereas ARNT alone did not significantly affect the VEGF promoter. Cotransfection of EPAS1 and ARNT synergistically increased VEGF promoter activity by 137-fold.

Analysis of the EPAS1 Transactivation Domain with C-terminal Deletion Mutants—Members of the basic helix-loop-helix/PAS family of transcription factors contain an activation domain in the C terminus (4, 27–32). To determine whether the EPAS1 C terminus is necessary for EPAS1 to transactivate the VEGF promoter through its HIF-1-binding site, we mutated the EPAS1 expression plasmid phEP-1 by 17-fold (Fig. 2A). This up-regulation of VEGF promoter activity by EPAS1 appears to be specific because cotransfection of the EPAS1 expression plasmid had no effect on endothelin-1 promoter or SV40 promoter activity (data not shown).

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Analysis of the EPAS1 Transactivation Domain with C-terminal Deletion Mutants—Members of the basic helix-loop-he-
VEGF promoter, we constructed several EPAS1 C-terminal deletion mutants. First, we confirmed the authenticity of the deletion mutants by sequencing and in vitro transcription and translation followed by SDS-polyacrylamide gel electrophoresis (Fig. 3A). The size of each in vitro translated protein was consistent with its predicted molecular mass. Each construct was then cotransfected with phARNT and pGL2hVEGF into BAECs. As shown previously, full-length EPAS1 (phEP-1-(1–870)) increased VEGF promoter activity by 127-fold. Deletion of the C terminus (phEP-1-(1–690)) increased transactivation activity further (Fig. 3B), and deletion of the 230 C-terminal amino acids (phEP-1-(1–639)) increased VEGF promoter activity by 567-fold. This increase raised the possibility of an inhibitory domain between amino acids 640 and 870 (see “Discussion”). Further deletion of the C terminus diminished transactivation (Fig. 3B), and deletion of the 385 C-terminal amino acids (phEP-1-(1–485)) abolished the stimulatory effect of EPAS1 completely (Fig. 3B), suggesting the presence of an activation domain between amino acids 486 and 639.

To exclude the possibility that deletion of the EPAS1 C terminus had affected the ability of EPAS1 to dimerize with ARNT, we performed electrophoretic mobility shift assays with in vitro translated full-length or truncated EPAS1 proteins and a labeled probe containing the VEGF HIF-1 site. Full-length EPAS1 or ARNT alone did not bind to the probe; DNA binding activity was detected only in the presence of both proteins (Fig. 3C). This DNA binding was specific to the HIF-1-binding site, abolished binding. Both C-terminal mutants, EPAS1-(1–639) and EPAS1-(1–485), formed heterodimers with ARNT and bound to the HIF-1-binding site (Fig. 3C). Thus, it appears that the failure of EPAS1-(1–485) to transactivate the VEGF promoter was not due to an inability to form heterodimers with ARNT or to bind to DNA.

Mapping the EPAS1 Transactivation Domain with Gal4-hEPAS1 Fusion Proteins—To map the transactivation domain of EPAS1 further, we generated a series of plasmids containing various fragments of EPAS1 fused to the DNA-binding domain (corresponding to amino acids 1–147) of the yeast transcription factor Gal4 (Fig. 4). The fusion plasmids were cotransfected into BAECs with a reporter construct containing five Gal4-binding sites in front of the minimal promoter of the adenovirus E1b gene and luciferase (pGL5Luc). The Gal4-hEPAS1 plasmid encoding EPAS1 amino acids 481–870 increased transcription by 146-fold relative to the plasmid encoding Gal4 DNA-binding domain alone (Gal4-(1–147)) (Fig. 4). Fusion plasmids encoding shorter EPAS1 C termini, Gal4-hEPAS1-(481–688) and Gal4-hEPAS1-(481–639), had higher transcriptional activity (9051- and 14,854-fold, respectively) (Fig. 4). Although further deletion of the C terminus diminished transactivation by EPAS1, Gal4-hEPAS1-(481–545) still had significantly higher transcriptional activity (2727-fold) than Gal4-(1–147) (Fig. 4). These results are consistent with those from the experiments with EPAS1 C-terminal deletion mutant plasmids (Fig. 3B) and indicate the presence of a potent activation domain between amino acids 481 and 639. Furthermore, the N-terminal part (amino acids 481–545) also appears to contain a transactivation domain that is transferable to the heterologous Gal4 protein (Fig. 4).

To determine whether the C-terminal portion of sequence 481–639 contains an activation domain, we deleted amino acids 481–639 from the N terminus. Transactivation diminished markedly with Gal4-hEPAS1-(540–639) (Fig. 4) and was only minimal with plasmids encoding amino acids 593–639 and 540–597. Thus, although the C-terminal portion of sequence 481–639 is required for maximal activity, it fails to confer activity on a heterologous protein. To rule out the possibility that this difference in activity reflected differences in the level of fusion protein expression, we performed parallel immunoblotting experiments with an anti-Gal4 DNA-binding domain monoclonal antibody. We found that the expression level for each Gal4 fusion protein was similar (data not shown). These data indicate that a potent transactivation domain is located within EPAS1 amino acids 481–545, although amino acids 546–639 are also necessary for full transactivation.

C-terminal Deletion Mutant Functions as a Dominant-negative Form of EPAS1—EPAS1-(1–485) was able to form heterodimers with ARNT and to bind to the HIF-1-binding site (Fig. 3C), but was not able to transactivate the VEGF promoter (Fig. 3B). Therefore, we hypothesized that phEP-1-(1–485) may function as a dominant-negative mutant. To test this possibility, we cotransfected into BAECs phARNT, phEP-1-(1–485), and phGL2hVEGF, phEP-1-(1–870) and phARNT (0.15 μg each in this experiment) increased VEGF promoter activity by 58-fold (Fig. 5A, white bars), phEP-1-(1–485) inhibited induction of the VEGF promoter by phEP-1-(1–870) and phARNT in a dose-dependent manner (Fig. 5A, white bars).

Hypoxia is an important stimulus for angiogenesis, and hy-
in a dose-dependent manner. This inhibition was nearly complete in desferrioxamine-treated cells was inhibited by phEP-1-(1–485) addition, induction of VEGF promoter activity by EPAS1 in hypoxia, we treated BAECs with desferrioxamine as described (4, 12, 34). Desferrioxamine increased the effect of EPAS1 on the VEGF promoter (460-fold maximum) (Fig. 5B). Desferrioxamine markedly increased VEGF mRNA abundance in control HUVECs infected by AdCMV (Fig. 7). However, this induction of VEGF mRNA by desferrioxamine was markedly inhibited in AdCMV.EPAS1-(1–485)-infected cells. These results suggest that dominant-negative EPAS1 functions as an inhibitor of endogenous VEGF gene induction under conditions that mimic hypoxia.

Because HIF-1α must dimerize with ARNT before activating target genes, we speculated that phEP-1-(1–485), by sequestering ARNT, may also work against HIF-1α as a dominant-negative protein. To test this hypothesis, we cotransfected into BAECs phARNT, the HIF-1α expression plasmid phHIF-1α, and increasing amounts of phEP-1-(1–485) with the reporter plasmid pGL2hVEGF (0.15 µg). Cells were treated with 130 µM desferrioxamine (DFX) for 24 h. For all constructs, pCAT3-Control was cotransfected to correct for differences in transfection efficiency. The -fold induction represents the ratio (mean ± S.E.) of normalized luciferase activity in cells treated with desferrioxamine to that in untreated cells.
We show in this report that EPAS1 induces VEGF gene expression in endothelial cells (Fig. 1) and that this induction is mediated, at least in part, at the transcriptional level (Fig. 2A). Although the activity of the VEGF promoter is regulated by multiple cis-acting elements, we (23) and others (15) have previously shown that the HIF-1 site located 1 kilobase distal to the transcription start site mediates hypoxia-induced increases in VEGF promoter activity. EPAS1-induced increases in VEGF promoter activity are mediated through an HIF-1-binding site and are enhanced markedly by cotransfection with ARNT (Fig. 2B). In two assay systems (Figs. 3 and 4), we identified a potent transactivation domain between amino acids 486 and 639. An EPAS1 mutant that lacks this transactivation domain functions as a dominant-negative form of the protein under basal normoxic conditions and after treatment with deferoxamine to pharmacologically mimic hypoxic conditions.

Both EPAS1 (Fig. 2B) and HIF-1α (1, 15) have been shown to bind the VEGF HIF-1 site. However, the tissue distribution of EPAS1 and HIF-1α is quite different; EPAS1 is mainly expressed in vascular endothelial cells, whereas HIF-1α is more ubiquitous. Thus, EPAS1 and HIF-1α may not necessarily compete for the same binding site in the same cells. Instead, they may collaborate to adapt tissue in response to hypoxia.

The EPAS1 transactivation domain between amino acids 481 and 545 is rich in acidic residues (25%; estimated pI 3.4). In its acidic nature, the EPAS1 activation domain is similar to those of other transcription factors such as Gal4 and Gcn4 (35). Jiang et al. (30) have reported that HIF-1α has two transactivation domains at amino acids 531–575 (TAD-N) and 786–826 (TAD-C). Sequence 481–545 of the EPAS1 activation domain and HIF-1α TAD-N share a high sequence identity (58%). In our study, however, we found that amino acids 546–639 of EPAS1 are also required for full target gene transactivation. This sequence is completely different from the sequence that follows TAD-N in HIF-1α. Although the most C-terminal sequence of EPAS1 (amino acids 820–870) does share a high sequence identity with HIF-1α-TAD-C (62%), sequence 820–870 did not function as a transactivation domain in our studies. This difference may be due to the presence of fewer acidic residues in the EPAS1 sequence in comparison with the HIF-1α sequence (12 versus 17%) or to a difference in the cell types tested.

The lower transcriptional activity of Gal4-hEPAS1 (481–870) in comparison with that of Gal4-hEPAS1 (481–639) (Fig. 4) raised the possibility that EPAS1 amino acids 640–870 contain a repressor domain. However, we were unable to demonstrate an inhibitory effect of Gal4-hEPAS1 (640–870) on adenovirus E1B gene minimal promoter activity (data not shown). An alternative explanation would be that removal of EPAS1 amino acids 640–870 may cause a conformational change that exposes the EPAS1 activation domain to the target gene.

A truncated EPAS1 protein lacking the C terminus retains the ability to form heterodimers and to bind to the HIF-1α-binding site (Fig. 3). Thus, it may sequester ARNT and prevent the formation of functional EPAS1/ARNT and HIF-1α/ARNT heterodimers in the way that Id inhibits formation of MyoD/E2A heterodimers (36). Indeed, the EPAS1 C-terminal mutant potentially inhibited induction of the VEGF promoter by wild-type EPAS1 and HIF-1α in the presence and absence of deferoxamine (Figs. 5 and 6). Furthermore, an EPAS1 C-terminal mutant inhibited the induction of endogenous VEGF mRNA by deferoxamine (Fig. 7). We have also found that EPAS1 induces the KDR/flk-1 promoter and that EPAS1 truncated at the C terminus inhibits induction of the KDR/flk-1 promoter by full-length EPAS1. As an EPAS1 dominant-negative protein, the C-terminal mutant may inhibit the transcription of VEGF as well as that of its receptor. The ability of the dominant-negative form of EPAS1 to inhibit the transcription of VEGF under hypoxic condition has important therapeutic implications, given the pivotal role of VEGF in pathological angiogenesis associated with many diseases (37–39).

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