Hypoxic Induction of the Hypoxia-Inducible Factor Is Mediated via the Adaptor Protein Shc in Endothelial Cells

Frank Jung,* Judith Haendeler,* Jörg Hoffmann, Agnes Reissner, Elisabeth Dernbach, Andreas M. Zeiher, Stefanie Dimmeler

Abstract—Tyrosine kinase cascades may play a role in the hypoxic regulation of hypoxia-inducible factor (HIF)-1. We investigated the role of tyrosine kinase phosphorylation and of the Shc/Ras cascade on hypoxic HIF-1 stabilization. Exposure of human umbilical vein endothelial cells to hypoxia results in HIF protein stabilization as early as 10 minutes, with a maximum at 3 hours, and also in Shc tyrosine phosphorylation, with a maximum at 10 minutes. To test whether Shc directly mediates hypoxia-induced HIF stabilization, human umbilical vein endothelial cells were transfected with a dominant-negative Shc mutant (dnShc), resulting in significantly reduced HIF protein levels compared with control. Similar results were obtained with cells transfected with dominant-negative Ras, a known downstream effector of Shc. Hypoxia-induced Ras activity was significantly reduced in cells transfected with dnShc compared with control levels, indicating that Ras indeed acts downstream from Shc. Moreover, cells pretreated with a specific Raf-1 kinase inhibitor, a known downstream effector of Ras, exhibited reduced HIF protein levels. To examine the functional consequences of Shc in hypoxic signaling, HIF-1 ubiquitination, protein stabilization, and endothelial cell migration were assessed. Overexpression of dnShc increased ubiquitination of HIF-1 and reduced the half-life of the protein. Moreover, dnShc, dominant-negative Ras, or the Raf-1 kinase inhibitor significantly inhibited migration under hypoxia. Thus, Shc in concert with Ras and Raf-1 contributes to hypoxia-induced HIF-1α protein stabilization and endothelial cell migration.

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Key Words: hypoxia ■ hypoxia-inducible factor-1 ■ Shc ■ Ras ■ endothelial cells

Hypoxia is an important regulatory stimulus for a variety of different biological processes, such as vascular tone and structure, angiogenesis, and erythropoiesis. These events are mediated by a variety of hypoxia-sensitive cellular proteins, which modulate cell-cell interaction and cell proliferation and differentiation. Many of these proteins, such as erythropoietin, vascular endothelial growth factor, the inducible NO synthase gene, or glycolytic enzymes, are under transcriptional control and are regulated in response to hypoxia. Hypoxia-inducible factor (HIF)-1, a transcription factor, is the O2-regulated subunit that determines its biological activity. The regulation of HIF is complex and involves not only changes on the transcriptional level but also posttranscriptional and posttranslational alterations in response to hypoxia. Perhaps most striking is HIF protein stabilization under hypoxic conditions and its rapid degradation on reoxygenation. Under normoxic conditions, HIF is subject to ubiquitination and proteasomal degradation. However, the regulatory step in hypoxia-mediated HIF regulation has not been determined. The von Hippel-Lindau tumor suppressor protein, a putative ubiquitin-protein ligase, appears to play a role in HIF protein stabilization, inasmuch as loss of the von Hippel-Lindau protein results in constitutive expression of the HIF protein. In addition, there is evidence that multiple domains in the HIF protein regulate its expression and that they do so by altering the ubiquitination of HIF-1α under nonhypoxic conditions. In addition to changes in the redox state affecting hypoxic signaling, changes in phosphorylation may influence HIF-1 expression and, eventually, the transactivation of genes. Indeed, hypoxic induction of HIF-1 is inhibited by pretreating cells with the tyrosine kinase inhibitor genistein or the serine/threonine phosphatase inhibitor sodium fluoride.

The adaptor protein Shc is an immediate substrate of tyrosine kinase and may play an important role in linking activated tyrosine kinase receptors to downstream hypoxic signaling, involving HIF protein stabilization. The Shc family of adaptor proteins consists of multiple protein-protein interaction domains: an amino-terminal phosphotyrosine binding domain, a central collagen homology domain, and a carboxy-terminal Src homology 2 domain. Shc exists in 3
isoforms of 46, 52, and 66 kDa and has been shown to be tyrosine-phosphorylated in association with the epidermal growth factor receptor.\textsuperscript{15} On activation, Shc is recruited to activated tyrosine kinase receptors, which then leads to phosphorylation at residues 239, 240, and 317 within the collagen homology domain.\textsuperscript{18–20} Thus, tyrosine-phosphorylated Shc is able to recruit Grb2/SOS, which ultimately results in activation of other downstream signaling components, eg, the Ras/extracellular signal–regulated kinase (ERK) cascade or Raf-1 kinase.\textsuperscript{15,16,18,21} Besides the involvement of tyrosine kinases and downstream effector pathways, the phosphatidylinositol 3-kinase (PI3-kinase) or MEK kinase also may play a role in the phosphorylation of HIF and subsequent protein stabilization or HIF-mediated gene transactivation.\textsuperscript{20,22–24}

In the present study, we investigated the role of different kinases and of the adaptor protein Shc in concert with Ras and Raf-1 on hypoxia-induced HIF-1 stabilization in human umbilical vein endothelial cells (HUVECs). Our results demonstrate that the Src kinase family, the tyrosine-phosphorylated adaptor protein Shc, and Ras/Raf-1 are critical for hypoxia-induced HIF-1 stabilization and inhibition of ubiquitination. Moreover, as a functional consequence, endothelial cell migration is reduced in cells transfected with a dominant-negative Shc mutant (dnShc) and a dominant-negative Ras mutant (dnRas) as well as in cells pretreated with a specific Raf-1 kinase inhibitor.

**Materials and Methods**

**Cell Culture**

HUVECs were purchased from Cell Systems/Clonetics and were cultured in endothelial basal medium as described.\textsuperscript{23} For studies involving hypoxic conditions, cells were purged with 95% N\textsubscript{2}/5% CO\textsubscript{2} for 20 minutes and then placed in an incubator (1% to 2% O\textsubscript{2}/5% CO\textsubscript{2}/balance N\textsubscript{2}) for 3 to 36 hours. Normoxia values were as follows: pH 7.31±0.1, PO\textsubscript{2} 119±2.3 mm Hg, and PCO\textsubscript{2} 44.3±2.4 mm Hg. Hypoxia values were as follows: pH 7.27±0.13, PO\textsubscript{2} 21.6±2.9 mm Hg, and PCO\textsubscript{2} 33.1±1.4 mm Hg.

**Protein Isolation and Western Blot Analysis**

For the detection of HIF-1 expression, HUVECs (4.0×10\textsuperscript{4} cells) were lysed in buffer as previously described.\textsuperscript{26} Proteins (30 μg/lane) were loaded onto SDS-polyacrylamide gels and blotted onto polyvinylidene difluoride membranes. Membranes were incubated as follows: primary antibodies (anti-HIF-1α from Transduction Laboratories, 1:250; anti-Shc from Transduction Laboratories, 1:1000; anti-phosphotyrosine from Upstate Technology, 1:500; and anti-myc from Santa Cruz, 1:250) at 4°C overnight and secondary antibody (anti-rabbit or anti-mouse 1:4000) for 1 hour. Enhanced chemiluminescence was performed according to the instructions of the manufacturer (Amersham). Blots were reprobed with actin (Roche, 1:2000) or tubulin (Neomarkers, 1:500), scanned, and semiquantitatively analyzed.

**Plasmids and Transfection**

dnShc (Y317F) and dnRas (S17N)\textsuperscript{27} were created by site-directed mutagenesis and cloned into a pcDNA3.1 vector (InVitrogen). Ubiquitin was cloned out of cDNA into a pcDNA3.1(–)myc/his vector as described previously.\textsuperscript{28} Transient transfection of HUVECs was performed by incubation of 3.7×10\textsuperscript{5} cells per 6-cm well with 3 μg plasmid as described previously.\textsuperscript{28}

**Half-Life Analysis**

HUVECs were incubated with 150 μmol/L CoCl\textsubscript{2} for 4 hours to induce hypoxia. After incubation with 10 μg/mL cycloheximide for 5, 15, 30, or 45 minutes, the half-life of HIF-1α was assessed by immunoblotting as described below. For the effect of the Src kinase family, the specific inhibitor PP1 was preincubated for 30 minutes.

**Immunoprecipitation**

HUVECs were plated as described above and starved in serum-free 1% BSA containing medium for 18 hours before exposure to hypoxia for the indicated times. Cells were then lysed in lysis buffer (in mmol/L: Tris-HCl 10 [pH 7.5], NaCl 150, KCl 2.5, Na\textsubscript{3}VO\textsubscript{4} 1, NaF 50, and glycerol phosphate 20) along with 0.5% Triton and 0.5% Nonidet P-40) for 30 minutes and scraped off the plates. After the cell debris was removed, protein extract was incubated with 5 μg anti-Shc antibody overnight. A/G Plus agarose (Santa Cruz) was then incubated at 4°C for 1 to 2 hours. Lysates were washed and loaded on SDS-PAGE.

**Detection of Ubiquitination**

HUVECs were cotransfected with vector and myc-tagged ubiquitin or with dnShc and myc-tagged ubiquitin. Hypoxia was simulated by the addition of 150 μmol/L CoCl\textsubscript{2} for 4 hours, and cells were incubated in the presence of 20 μmol/L MG132, the specific inhibitor of the ubiquitin proteasome complex, to maintain ubiquitinated HIF-1α. Cells were then lysed in lysis buffer (as described above) containing 10 mg/mL N-ethylmaleimide for 30 minutes and scraped off the plates. After the cell debris was removed, protein extract was incubated with 5 μg anti-myc antibody overnight. A/G Plus agarose (Santa Cruz) was then incubated at 4°C for 1 to 2 hours. Lysates were washed and loaded on SDS-PAGE.

**Activated Ras Interaction Assay**

The activated Ras interaction assay was performed as described previously.\textsuperscript{29} Briefly, the Raf-1–RBD construct was transformed into Escherichia coli. Fusion proteins were affinity-purified on glutathione-Sepharose 4B to obtain the glutathione S-transferase–RBD domain. Beads were used to precipitate Ras-GTP from lysates of HUVECs exposed to hypoxia for 10 and 30 minutes.

**Migration Assay**

The migration assay was performed as previously described.\textsuperscript{30} Briefly, cells were transfected, and plates were scratched by using a rubber policeman. The distances between cell borders were measured by using light microscopy before and after exposure of the cells to normoxia or hypoxia for 36 hours.

**Statistical Analysis**

Data are expressed as mean±SEM from at least 3 independent experiments. Statistical analysis was performed with ANOVA for multiple variables and with t tests for comparison of 2 groups with normal distribution. In the case of nonnormal distribution, a nonparametric Wilcoxon test was used. All statistical analyses were performed with SPSS for Windows 7.0.

**Results**

**Effect of Hypoxia on HIF-1α and Shc Protein Expression in HUVECs**

To determine the effect of hypoxia on HIF-1α expression, HUVECs were exposed to normoxia and hypoxia between 5 minutes and 3 hours. Exposure to hypoxia resulted in a significant increase of HIF-1α protein levels as early as 10 minutes, with a maximum at 3 hours (Figure 1A). Because the adaptor protein Shc plays an important role as a signaling molecule after tyrosine kinase activation, we investigated a potential role of Shc as an upstream effector of hypoxia-induced HIF-1α protein stabilization. To determine whether
Shc is tyrosine-phosphorylated under hypoxic conditions, HUVECs were exposed to hypoxia, and Shc was immunoprecipitated. Western blot analysis using anti-phosphotyrosine antibodies revealed significantly increased tyrosine phosphorylation of the p52 Shc protein in hypoxic cells, with a maximum at 10 minutes, compared with normoxic cells, which returned to baseline 30 minutes after exposure (Figure 1B). The increase in Shc tyrosine phosphorylation was not due to enhanced Shc protein levels under hypoxic conditions, as shown in Figure 1C. To test whether p52Shc directly associates with HIF-1α under hypoxic conditions, Western blotting was performed with the use of HIF-1α antibodies. However, no direct association was observed between Shc and HIF-1α at any time point (Figure 1D, top).

Hypoxia-Induced HIF Stabilization Is Mediated via the Adaptor Protein Shc and Its Downstream Effector Ras

Having demonstrated that hypoxia-induced Shc tyrosine phosphorylation precedes hypoxia-induced HIF-1α protein stabilization, we overexpressed dnShc in HUVECs to demonstrate that Shc functions as an upstream regulator of hypoxia-induced HIF-1α protein stabilization. dnShc significantly reduced hypoxia-induced HIF-1α protein stabilization (Figure 2A). Inasmuch as hypoxia-induced Shc tyrosine phosphorylation peaks as early as 10 minutes and HIF protein stabilization is maximal at 3 hours, other intermediate signaling molecules may play a role in the signaling cascade downstream from Shc, leading to HIF protein stabilization. Therefore, Ras, the known Shc downstream effector, was investigated. Overexpression of dnRas inhibited hypoxia-induced HIF-1α protein stabilization to an extent similar to that found with dnShc (Figure 2A). To further demonstrate that Ras acts downstream from Shc, we performed an activated Ras interaction assay by using HUVECs transfected with dnShc or a control plasmid. Affinity-precipitated Ras showed maximal Ras activity 30 minutes after hypoxic exposure in vector-transfected cells (Figure 2B). dnShc completely abrogated hypoxia-induced Ras activity. These data suggest that hypoxic induction of HIF protein stabilization may initially be mediated via the adaptor protein Shc and its downstream effector Ras.

Raf-1 Kinase Acts Downstream From Shc and Ras, Mediating Hypoxia-Induced HIF Stabilization

Ras has been shown to act via activation of Raf-1. Therefore, cells were preincubated with 5-iodo-3-[(3,5-dibromo-4-hydroxyphenyl)methylene]-2-indolinone, a specific Raf-1 kinase inhibitor, before hypoxic exposure. Exposure to hypoxia significantly reduced HIF-1 protein stabilization in the presence of the Raf-1 kinase inhibitor (34±14.2% inhibition) (Figure 3A). These data suggest that hypoxia-induced HIF-1α stabilization is mediated, at least in part, via the Raf-1 kinase effector pathway. To further analyze the contribution of potential candidates, we investigated the involvement of ERK1/2, p38 kinase, the PI3-kinase pathway, or mTOR16 by using the specific MEK inhibitor PD98059, the p38 kinase inhibitor SB203580, the PI3-kinase inhibitor Ly294002, and...
rapamycin, respectively. However, PD98059, SB203580, and Ly294002 revealed no effect on HIF-1 protein stabilization under hypoxic conditions (Figure 3B). Rapamycin showed a slight inhibition of HIF-1α protein stabilization (Figure 3B). This could be well explained, inasmuch as mTOR controls the mammalian translation machinery via activation of the p70s6k protein kinase and via inhibition of the eIF4E inhibitor 4E-BP1.32 Thus, inhibition of mTOR by rapamycin could directly result in reduced protein expression and, therefore, also in reduced induction of HIF-1α protein levels. These data suggest that hypoxia-induced increases in HIF-1α protein levels appear to involve predominantly the Ras–Raf-1 signaling pathway.

Role of Src Kinase Family in Hypoxia-Induced HIF-1α Stabilization

To gain insight regarding a potential upstream tyrosine kinase, we investigated the effect of the specific Src kinase

Figure 2. A, Effect of overexpression of dnShc and dnRas on hypoxia-induced HIF protein stabilization. HUVECs were transfected with dnShc (dom. neg. Shc) or dnRas (dom. neg. Ras) as well as a control plasmid (control) before exposure to normoxia and hypoxia for 3 hours. Protein was prepared, and Western blot analysis was performed with the use of anti–HIF-1α. Data are mean±SEM (n=3). P<0.05 for dnShc and dnRas vs control. B, Effect of overexpression of dnShc on hypoxia-induced Ras activity. HUVECs were transfected with dnShc or control plasmid (control) and exposed to hypoxia for 10 minutes and 30 minutes. Affinity-precipitated Ras was detected by Western blotting with the use of anti-Ras antibodies. Data are mean±SEM (n=3). P<0.04 for cells transfected with dnShc vs control after 30 minutes of hypoxic exposure.

Figure 3. A, Effect of 5-iodo-3-[(3,5-dibromo-4-hydroxyphenyl)methylene]-2-indolinone, a specific Raf-1 kinase inhibitor, on hypoxia-induced HIF protein stabilization. HUVECs were preincubated with or without a specific Raf-1 kinase inhibitor (500 nmol/L) before hypoxic and normoxic exposure for 3 hours. Data are mean±SEM (n=3). P<0.05 for cells pretreated with the Raf-1 inhibitor vs control cells after 3 hours of hypoxic exposure. B, Cells were also preincubated with the specific MEK inhibitor PD98059 (10 μmol/L), the p38 kinase inhibitor SB203580 (10 μmol/L), or the PI3-kinase inhibitor Ly294002 (10 μmol/L) under hypoxic or normoxic conditions for 3 hours. Western blot analysis was performed with the use of anti–HIF-1α. Data are mean±SEM (n=3).
family inhibitor, PP1, on HIF-1α half-life. Hypoxia was induced with 150 μmol/L CoCl₂ for 4 hours, and the half-life of HIF-1α was assessed after treatment with cycloheximide for 15, 30, and 45 minutes. As demonstrated in Figure 4A, compared with the control condition, PP1 reduced the half-life of hypoxia-induced HIF-1α protein (half-life of control cells, 33.0±0.0 minutes; half-life of cells treated with PP1, 22.5±3.1 minutes; P<0.05). These data demonstrate a role for the Src kinase family in HIF-1α protein stabilization. Recently, it has been reported by Chen et al. that in ﬁbroblasts, tyrosine phosphorylation of the platelet-derived growth factor (PDGF) receptor is involved in hypoxia-induced HIF-1α activation. Therefore, we investigated the effect of the speciﬁc PDGF receptor kinase inhibitor, AG 1296, on hypoxia-induced HIF-1α protein levels. However, incubation with AG1296 did not inhibit hypoxia induced HIF-1α protein levels (Figure 4B).

**dnShc Inhibited Half-Life and Induced Ubiquitination of HIF-1α**

To gain further insight into the role of Shc on the half-life of HIF-1α, we transfected HUVECs with dnShc. As demonstrated in Figure 2A, dnShc reduced the hypoxia-induced HIF-1α protein levels. The remaining HIF-1α protein showed a shorter half-life compared with vector-transfected cells (half-life of vector-transfected cells, 25.6±5.5 minutes; half-life of dnShc-transfected cells, 12.5±3.1 minutes; P<0.05) (Figure 5A). Furthermore, we cotransfected HUVECs with vector and myc-tagged ubiquitin or with dnShc and myc-tagged ubiquitin. Hypoxia was induced by using CoCl₂ for 4 hours; to maintain the ubiquitinated HIF-1α, cells were treated with the speciﬁc proteasome complex inhibitor MG132. Immunoprecipitations were performed with an anti-myc antibody or an anti-Shc antibody, and in both cases, HUVECs transfected with dnShc revealed more ubiquitinated HIF-1α than did vector-transfected cells, demonstrating an important role for Shc in hypoxia-induced HIF-1α protein ubiquitination and subsequent degradation (Figure 5B and data not shown).

**Hypoxia-Induced Endothelial Cell Migration Is Mediated via the Adaptor Protein Shc and Its Downstream Effectors Ras and Raf-1**

Hypoxia-induced endothelial cell migration may be an important requisite for angiogenesis. A variety of speciﬁc genes, such as vascular endothelial growth factor, may participate in the regulation of this process, and they may function under the control of the transcription factor HIF-1α. Therefore, to address a functional consequence of hypoxia-induced Shc and Ras activation, we assessed endothelial cell migration. HUVECs were transiently transfected with dnShc, dnRas, or a control plasmid and preincubated with the speciﬁc Raf-1 inhibitor, 5-iodo-3-[(3,5-dibromo-4-hydroxyphenyl)methylene]-2-indolinone. Hypoxia-induced endothelial cell migration was signiﬁcantly reduced in cells transfected with dnShc and dnRas and in cells pretreated with the Raf-1 kinase inhibitor (Figure 5C). These data suggest that endothelial cell migration in response to hypoxia is mediated, at least in part, via the Shc/Ras cascade.

**Discussion**

The results of the present study demonstrate that hypoxia induces HIF-1α protein stabilization as early as 10 minutes, with a maximum at 3 hours. Hypoxic signaling leading to HIF protein stabilization is mediated via the Shc/Ras signaling cascade, as demonstrated by overexpression of dnShc and dnRas in HUVECs. Hypoxia-induced tyrosine phosphorylation of Shc seems to occur as one of the earliest events, peaking at 10 minutes after hypoxic exposure, followed by subsequent Shc-dependent activation of Ras, which showed maximal activity under the inﬂuence of hypoxia at 30 minutes.

Previous studies have suggested that hypoxic signaling leading to HIF expression involves activation of tyrosine
kinases, because induction of HIF-1 was inhibited by pretreating cells with the tyrosine kinase inhibitor genistein.\textsuperscript{14}

However, a specific tyrosine kinase initiating the signaling events leading to HIF stabilization has not been identified. It has been shown that the transformation of cells with the constitutively active v-Src oncogene results in increased HIF-1\textalpha protein expression, HIF-1 DNA binding activity, and gene transactivation,\textsuperscript{35} suggesting activation of an intracellular tyrosine kinase under hypoxic conditions. Indeed, Seko et al\textsuperscript{35} have shown that c-Src is induced in cardiac myocytes under hypoxic conditions. However, c-Src-deficient mouse embryonic fibroblasts demonstrate normal HIF-1 protein induction and DNA binding activity under the influence of hypoxia, thereby excluding a major role for c-Src, ie, mediation of hypoxia-induced HIF-1\textalpha stabilization.\textsuperscript{34,36} Our findings now demonstrate a role for the Src kinase family in hypoxia-induced HIF-1\textalpha protein levels, inasmuch as the specific inhibitor PP1 reduced the half-life of HIF-1\textalpha. This is in accordance with data demonstrating that tyrosine phosphorylation and, thereby, activation of Shc is dependent on the Src kinase family.\textsuperscript{37,38} Moreover, the adaptor protein Shc is recruited on a variety of different membranous signals to activate membranous tyrosine kinase receptors, where it is phosphorylated, allowing association with the GRB-2/SOS complex.\textsuperscript{15,16,18,21} However, our data exclude the involvement of one of the receptor tyrosine kinases, namely, the PDGF receptor tyrosine kinase. Our data demonstrate that Shc is tyrosine-phosphorylated in endothelial cells under hypoxic conditions, whereas Shc protein expression is not altered. However, the finding that Shc does not directly associate with HIF suggests the presence of other signaling intermediates. Association of Shc with the GRB-2/SOS complex can ultimately result in the activation of the downstream effector Ras-ERK pathway.\textsuperscript{39} Indeed, in the present study, we were able to demonstrate that transfection with dnRas acting downstream from Shc results in decreased HIF-1 protein stabilization, which strongly suggests Ras as an intermediate signaling partner in the hypoxia-induced link between Shc and HIF expression. This is in accordance with a study from Chen et al,\textsuperscript{22} who demonstrated increased HIF protein levels in Ras-transfected cells.

Previous studies have demonstrated that hypoxia-activated ERK1 is able to directly phosphorylate HIF-1, leading to enhanced transcriptional activity of HIF-1,\textsuperscript{23,24} without affecting protein stability.\textsuperscript{40} Thereby, the Ras-dependent mitogen-activated protein kinase appears to specifically upregulate the transactivation activity of HIF-1\textalpha through direct phosphorylation of its regulatory inhibitory domain.\textsuperscript{41} In addition, it has been proposed that the PI3-kinase–Akt pathway may promote HIF-1\textalpha protein stability via inhibition of its downstream effector GSK-3, which targets proteins for degradation.\textsuperscript{41} However, in the present study, preincubation of cells with the
MEK inhibitor PD98059 or the PI3-kinase inhibitor Ly294002 did affect hypoxia-induced HIF-1α protein stabilization. Thus, although our data do not preclude the possibility of direct phosphorylation of HIF through the mitogen-activated protein kinase or the PI3-Akt kinase pathway and, thereby, an increased transcriptional activity of HIF, these kinases do not appear to play a major role in the observed HIF protein stabilization on hypoxic induction.

Raf-1 has been reported to be able to act as a downstream effector of Ras independent of ERK.42 Indeed, our data indicate that Raf-1 is involved in hypoxic HIF-1α protein stabilization, although the mechanism is unclear. Raf-1 could possibly influence HIF protein stabilization via the ubiquitin proteasome complex. It is known that Raf-1 can interact with the ubiquitin proteasome complex, leading to phosphorylation and degradation of IκBα, which then leads to nuclear factor-κB stabilization and activation.43,44 Consistent with this concept, overexpression of dnShc increased HIF-1α ubiquitination and subsequently reduced the protein half-life of HIF-1α. Ischemia of myocardium and other tissues can lead to new vessel formation.45,46 Endothelial cell migration plays an important role in the initial phase of angiogenesis. However, to date, there are no reports suggesting a role for Shc-mediated signaling in hypoxia-induced endothelial cell migration. Therefore, we used hypoxia-dependent endothelial cell migration as a functional readout to demonstrate a potential influence of the Shc-Ras-Raf pathway on the functional consequence of HIF regulation. Endothelial cell migration was significantly reduced in cells transfected with dnShc and with dnRas compared with cells transfected with control plasmid. Shc and focal adhesion kinase are known to play a role in growth factor–mediated and integrin-mediated cell migration,47–49 which may support our data that hypoxia-mediated endothelial cell migration is mediated via Shc signaling.

In conclusion, we provide evidence that the adaptor protein Shc and its downstream effectors, Ras and Raf-1, can mediate hypoxia-induced HIF-1 stabilization in endothelial cells and contribute to the complex hypoxia transduction pathway from the putative oxygen sensor to the activation of HIF.

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