Hypoxia and HIF-1α Stability
Another Stress-Sensing Mechanism for Shc

Jun-ichi Abe, Bradford C. Berk

The formation of new blood vessels after ischemia and vascular injury is a critical process for tissue repair. A complex interplay of stress-activated signal transduction pathways is activated in response to ischemia and injury to initiate the repair process. Among these pathways, the hypoxia-inducible transcription factor-1 (HIF-1α) plays an essential role by transactivating the VEGF gene and activating a pattern of gene expression associated with mobilization, migration, and recruitment of endothelial cells and smooth muscle cells to form new blood vessels.1–3 The report by Jung et al4 in this issue of Circulation Research investigates the signaling pathway responsible for hypoxia-induced HIF-1α protein stabilization and concludes that a mechanism involving Src, Shc, Ras, and Raf-1 is critical in endothelial cells.

In this editorial, we discuss 5 key issues raised by the work of Jung et al.4 (1) Signal transduction pathways that regulate protein stabilization are important pathophysiological mechanisms that may provide novel therapeutic approaches. (2) Shc isoforms represent a family of stress-regulated proteins that act as sensors for redox, oxygen species (ROS), and other genotoxic stresses. (3) Shc proteins mediate signal transduction in localized subcellular compartments, which are likely essential for hypoxia signaling pathways. (4) Cell migration in response to hypoxia likely involves multiple mechanisms including Shc. (5) Dominant-negative (DN) proteins represent an important technical advance to define signal transduction pathways, but appropriate controls must be used to avoid nonspecific effects.

Structure of Shc Isoforms and Grb2/Sos/Ras
Shc is an adaptor protein that possesses no intrinsic enzymatic activity, yet alterations in its structure have profound functional consequences as shown by the fact that Shc was first identified as a protooncogene.5 The mammalian SHC locus encodes three different isoforms with molecular masses of 52 kDa (p52Shc), 46 kDa (p46Shc), and 66 kDa (p66Shc). These isoforms share a Src-homology2 (SH2) domain, a collagen-homology (CH1) region, and a phosphotyrosine-binding (PTB) domain (Figure 1). Shc becomes tyrosine-phosphorylated upon cell stimulation by many growth factors including EGF.6 Upon binding of EGF to the EGF receptor, the activated EGF receptor tyrosine kinase phosphorylates Shc. Next, binding of the Shc PTB domain to the autophosphorylated EGF receptor occurs, and this binding seems to play a key role for efficient tyrosine phosphorylation of Shc. Upon tyrosine phosphorylation, Shc now forms a complex with Grb2-Sos via binding of the SH2 domain of Grb2 to phosphotyrosine residues Tyr239, Tyr240, and Tyr317 of Shc.5

Grb2 is composed of one SH2 domain and two SH3 domains.7 The two SH3 domains of Grb2 bind to proline-rich residues near the carboxyl terminus of Sos, a Ras guanine nucleotide exchange factor. Upon cell stimulation with growth factors, Grb2 through its SH2 domain binds to Shc, and this leads to translocation of Sos to the plasma membrane where Ras is located. Sos then increases the exchange of GDP for GTP on Ras. The GTP-bound active form of Ras then triggers the activation of the MAP kinase cascade and stimulation of ERK1/2.8

Signal Transduction Pathways That Regulate Protein Stabilization
The major finding of the present study by Jung et al4 is that Shc and Ras mediate hypoxia-induced stabilization of HIF-1α. However, the mechanism is unusual in that ERK1/2 is not responsible for the effect on protein stability because the MEK1 inhibitor PD98059 did not prevent stabilization. This is in agreement with previous studies by Pouysségur and coworkers9 who showed that ERK1/2 phosphorylated HIF-1α and increased its transcriptional activity, but did not change protein expression. The present study suggests that a Shc-Ras-Raf-1–mediated pathway is responsible for HIF-1α stabilization by demonstrating that a Raf-1 inhibitor caused HIF-1α degradation. It appears likely that phosphorylation of transcription factors, ubiquitination, and trafficking to the 26S proteasome is an important feedback regulatory mechanism for stress responses such as hypoxia. For example, stability of the TAL1/SCL transcription factor in endothelial cells is regulated by ERK1/2-dependent phosphorylation of serine 122.10 These results suggest that a therapeutic approach to modulate angiogenesis stimulated by hypoxia is to target the kinases, ubiquitin-conjugating enzymes, and ligases involved in protein stability.

p66Shc as a Stress-Response Mediator
An important role for p66Shc in the cellular stress response, especially to ROS, was first proposed by Migliaccio et al.11 They reported that ablation of p66Shc enhanced cellular resistance to apoptosis induced by H2O2 or UV light, and targeted disruption of the mouse p66Shc gene induced stress

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resistance and prolonged life span. Migliaccio et al also showed a critical role for serine phosphorylation of p66Shc, because they found that mutation of serine 36 (p66Shc-S36A) failed to restore the normal stress response in p66Shc−/− cells. Recently, Nemoto et al demonstrated that p66Shc serine 36 phosphorylation regulated intracellular oxidant levels and negatively regulated activity of the transcription factor forkhead.

An important mechanism linking the stress response, Shc, and HIF-1α is the ability of HIF-1α to associate with the tumor suppressor protein p53 and prevent p53 degradation. In HIF-1α−/− embryonic stem cells, hypoxia is unable to induce p53 expression, indicating that p53 is regulated by HIF-1α. p66Shc has also been reported to regulate p53 and p21 expression in cells exposed to oxidative stress. Thus, it is possible that an important aspect of Shc regulation of HIF-1α stability is the effect on p53 and p21 expression and function. Importantly, it seems that serine phosphorylation of Shc mediates these effects.

**Shc Isoforms as Regulators of Receptor and Redox Compartmentalization**

Although all three Shc isoforms share similar structures (Figure 1) and are tyrosine-phosphorylated upon activation by growth factors, it has been reported that the three isoforms have distinct physiological roles. For example, p66Shc and p46Shc/p52Shc exert opposing effects on the c-fos promoter, and p66Shc alone has been reported to act as a negative regulator of EGF-stimulated ERK1/2 activity. Shc was then phosphorylated by redox-sensitive kinases in a location-specific manner and acts as a negative regulator of EGF-stimulated ERK1/2 activity. In particular, individual Shc isoforms seem to play important roles in receptor crosstalk and endocytosis in a redox-dependent manner. Indeed, our laboratory found that p66Shc uniquely associated with the PDGF receptor (PDGFβ-R) in vascular smooth muscle cells and defined two different receptor pools. One population of PDGFβ-R exists in which Shc is bound to the receptor in the basal state, whereas in the other population the PDGFβ-R is not complexed to Shc. Transactivation of PDGFβ-R by G protein–coupled receptors was limited to receptors bound to Shc in a redox-dependent manner. These data suggest that an important function of Shc isoforms may be to compartmentalize subcellular signal transduction. Because alterations in the concentration of ROS and oxygen are highly localized to specific cell compartments, phosphorylation of Shc by redox-sensitive kinases in a location-specific manner may be a critical component of the hypoxia-induced signal transduction described in the present study.

**Cell Migration and Shc**

Jung et al report that a Shc-Ras signaling pathway regulates endothelial cell migration, probably as a result of HIF-1α protein stabilization. Previously, Gu et al reported that Shc and FAK regulated cell migration through two different mechanisms: one is a pathway from Shc through the MAP kinase pathway leading to the stimulation of random cell motility, and the other is from FAK through p130Cas leading to stimulation of directionally persistent cell migration. The directional migration induced by FAK and p130Cas correlates with more extensive, oriented actin microfilament bundle organization and focal contact formation, which may be more important in angiogenesis. These results suggest that it will be important to define the effect of DN-Shc and DN-Ras on FAK and p130Cas signaling.

**Dominant-Negative Mutants as Signal Transduction Tools**

In the present study, the authors show a critical role for Shc in hypoxia-mediated regulation of HIF-1α expression by using a dominant-negative Shc (DN-Shc) in which tyrosine 317 was mutated to phenylalanine (Y317F). Previously, Wary et al characterized this same mutant and showed that the inhibitory mechanism is to prevent Grb2 binding. Wary et al described a Shc-dependent signaling pathway in which fibronectin-mediated integrin activation caused Fyn activation and binding of Fyn (via its SH3 domain) to Shc (via Shc proline-rich region, Figure 2A). Shc was then phosphorylated at Y317 and recruited Grb2, thereby stimulating the Ras-ERK1/2 pathway. DN-Shc (Y317F) inhibited fibronectin-induced Shc-Grb2 association and prevented ERK1/2 activation (Figure 2B). It is important to emphasize that DN-Shc (Y317F) acts as a trapping mutant, meaning that when it is overexpressed it “outcompetes” endogenous Shc for binding to signaling mediators such as Fyn and other SH3 binding proteins (Figure 2). Because Y317F Shc does not have a Grb2 binding site, it inhibits Grb2 signaling by active Fyn (Figure 2C). This mechanism of inhibition differs from that associated with mutation of the active site of proteins such as kinases (eg, DN-Fyn or DN-ERK2), which act as more specific inhibitors. Specifically, three other mechanisms of DN-Shc (Y317F) inhibition of signaling need to be considered. (1) Shc is not the only molecule that can associate with the SH3 domain of Fyn, so it is possible that overexpression of DN-Shc “traps” the Fyn SH3 region and prevents interaction with another Fyn binding partner (Figure 2D, right). This mechanism can be evaluated by determining the effect of Shc wild-type overexpression, which may inhibit in a “dose-dependent” manner. (2) Other SH3 binding proteins besides Grb2 could be bound by DN-Shc preventing downstream signaling (Figure 2D, left). (3) Shc is also serine- and threonine-phosphorylated in response to many agonists sug-
gesting another pathway for regulation. For example, Okada et al.\textsuperscript{15} reported that p66\textsuperscript{Shc} serine and threonine phosphorylation regulates its association with the tyrosine-phosphorylated EGF receptor. There is no information regarding the effects of Y317F mutations on Shc serine-threonine phosphorylation. In summary, the present study defines a novel role for Shc in the cellular response to stress and characterizes a signaling pathway for stabilization of HIF-1\textalpha protein. Despite the present advance, it is clear that many critical questions remain unanswered in our quest to understand the signaling pathways responsible for cellular responses to hypoxia.

**Figure 2.** Dominant-negative mechanisms for adaptor proteins like Shc. Shown (A through C) is presumed mechanism of present study based on inhibition of Grb2. Shown (D) is alternative mechanism induced by competing (D, right) or association with other SH3 binding proteins besides Grb2 (D, left).

**References**


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