Subject Review

The Ubiquitin Ligase Siah2 and the Hypoxia Response

Koh Nakayama,[2] Jianfei Qi,[1] and Ze'ev Ronai[1]

[1]Signal Transduction Program, Burnham Institute for Medical Research, La Jolla, California and [2]Medical Top Track Program, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan

Abstract

Growing evidence indicates that ubiquitin ligases play a critical role in the hypoxia response. Among them, Siah2, a RING finger ligase, is an important regulator of pathways activated under hypoxia. Siah2 regulates prolyl hydroxylases PHD3 and 1 under oxygen concentration of 2% to 5%, thereby allowing accumulation of hypoxia-inducible factor (HIF)-1α, a master regulator of the hypoxia response within the range of physiological normoxic to mild hypoxic conditions. Growing evidence also indicates an important function for Siah2 in tumor development and progression based on pancreatic cancer, mammary tumor, and melanoma mouse models. This review summarizes our current understanding of Siah2 regulation and function with emphasis on hypoxia and tumorigenesis. (Mol Cancer Res 2009;7(4):443–51)

Introduction

Organisms respond to decreased oxygen (hypoxia) by altering fundamental physiological activities such as respiration and metabolism to maintain homeostasis. Without a proper response to hypoxia, multiple systems could become deregulated resulting in potential cell death. Organisms from fungi or yeast to mammals can mobilize a hypoxia response. However, biological responses differ from species to species. Fungi mostly control their metabolism to alter growth, whereas mammals have acquired more complex responses in accordance with their need to maintain multiple systems. In humans, the hypoxia response can include physiological responses ranging from altered metabolism, respiration, blood cell production, and neovascularization (1).

Two cellular responses to hypoxia are possible: an acute/transient hypoxia response or a chronic/sustained response. In an acute response, cells immediately begin conserving ATP by limiting the cellular processes consuming energy and decrease the O_2 consumption. They also increase respiration and produce specific cytokines for the cell survival, migration, or vascularization. During chronic hypoxia, increases in red blood cell number or formation of new vasculature occur, adapting an organism to decreased oxygen conditions. Normally, cells in our body exist at around 5% oxygen concentration. Although it is lower than the oxygen concentration in the atmosphere (21%), which is generally referred to as normoxia, we will refer to the range of 5% to 2% oxygen as "physiological normoxia to mild hypoxia conditions" in this review. Cells have developed highly sensitive mechanisms to detect subtle decreases (e.g., from 5% to 2%) in oxygen levels in order to trigger the hypoxia to response. Tumorigenic environments also create a state of chronic hypoxia. As cancer cells grow, hypoxic regions with insufficient connections to the vascular network form. If this condition persists, a hypoxia response is induced, resulting in production of cytokines such as vascular endothelial growth factor (VEGF), which promote vascularization. Eventually a tumor establishes connection to the vascular network, enhancing tumor cell growth and promoting metastasis (recently reviewed in refs. 2, 3). In this review, we focus on activity of the ubiquitin ligase Siah2 in regulating the hypoxia response and discuss its role in tumor development.

The Hypoxia-Inducible Factor Pathway

Role of the Transcription Factor Hypoxia-Inducible Factor in Hypoxia Response

Cells exposed to decreased oxygen alter fundamental cellular activities, such as translation or transcription. Hypoxia-inducible factor (HIF) transcription factors are key regulators of the latter. HIFs are basic helix-loop-helix/PAS proteins consisting of an α subunit (e.g., HIF-1α) and a β subunit (e.g., ARNT). Together they form a heterodimer that regulates transcription by binding to a hypoxia-responsive element (HRE) (refs. 3, 4). HRE has a core five-nucleotide sequence RCGTG (R: A/G), which is well conserved among numerous hypoxia-responsive genes. Those targets can be categorized into several groups on the basis of their physiological roles, such as cell cycle (cyclin, ref. 5; IGF2, ref. 6; TGF-α, ref. 7); cell death (NIP3, NIX, refs. 8, 9); metabolism (GLUT1, ref. 10; PGK-1, refs. 11, 12; G3PDH, ref. 13; PDK1, refs. 14, 15); angiogenesis (VEGF, refs. 16-18); erythropoiesis (EPO, ref. 19); and cell migration (c-met, ref. 20; CXCR4, ref. 21). As many of these genes function in the hypoxia response, HIF is considered as a central regulator of hypoxia.

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Requests for reprints: Koh Nakayama, Tokyo Medical and Dental University, Yushima 1-5-45, Tokyo 113-8510, Japan. Phone: 81-3-5803-4815; Fax: 81-3-5803-4815. E-mail: nakayama.mtt@mri.tmd.ac.jp or Jianfei Qi, Signal Transduction Program, Burnham Institute for Medical Research, La Jolla, CA, 92130. E-mail: jfqi@burnham.org

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Of the three HIF-α proteins (1α, 2α, and 3α), HIF-1α and HIF-2α are particularly critical for the hypoxia response; recent reports show a distinct function of HIF-1α and HIF-2α such as transcriptional regulation in cooperation with c-Myc on cancer progression (22, 23). The role of HIF-3α is less well understood, but it has been suggested that the alternative spliced form of HIF-3α binds to and inhibits transcriptional activity of HIF-1α (24). Although HIF-1α and HIF-2α share several common targets such as VEGF, it has recently been shown that Oct4 is a specific, direct target of HIF-2α but not of HIF-1α (25). Moreover, it is suggested that HIF-1α functions in early stages of neuroblastoma development, whereas HIF-2α promotes growth and metastasis in later stages (23). Expression patterns of these two genes also differ: HIF-1α is expressed ubiquitously, whereas HIF-2α expression is limited to endothelium, kidney, lung, heart, and small intestine (22). Finally, in mice, knockout of either gene results in an embryonic lethal phenotype. However, embryos die of different causes: HIF-1α embryos die around E10.5 due to cardiac and vascular defects (26-28), whereas HIF-2α null embryos die of bradycardia, vascular defects, and incomplete lung maturation (29-32).

Regulation of HIF-α in Normoxia

HIF-1α and HIF-2α are similarly regulated by oxygen levels. In decreased oxygen concentrations, HIF-α levels increase mainly because of regulation at the protein level. In normoxia, HIF-α subunits are actively ubiquitinated and degraded by the proteasome. The E3 ligase responsible for this ubiquitination is a Skp, Cullin, F-box (SCF)-type ubiquitin ligase complex consisting of pVHL, elongin B, C (also called the VBC complex), and Rbx1 (33, 34). pVHL is the molecule recognizing the HIF-α subunit (35). Mutation of pVHL is seen in patients with renal clear cell carcinoma. pVHL/HIF-α interaction requires hydroxylation of HIF-α prolines 402 and 564 located in what is termed the oxygen-dependent degradation domain (ODD; refs. 36, 37). Once hydroxylated, HIF-αs are captured by the pVHL binding pocket and ubiquitinated with subsequent degradation (38, 39).

HIF-1α undergoes other posttranslational modifications. It can be phosphorylated by ERK-MAPK, resulting in increased transcriptional activity (40). HIF-1α can also be SUMOylated, which can either positively or negatively regulate its stability (41-43). Hydroxylation of HIF-1α asparagine 803, located in the C-terminal transcriptional activation domain, inhibits transcriptional activity by blocking recruitment of the coactivators p300/CBP (44).

Hydroxylation by PHD

HIF-α is hydroxylated on prolines 402 and 564 by a family of enzymes called prolyl hydroxylase domain-containing proteins or PHDs (EGLN, HPH) and on asparagine 803 by FIH (factor inhibiting HIF; refs. 37, 44). Although they hydroxylate different residues, PHD and FIH belong to the Fe(II) and 2-oxoglutarate-dependent oxygenase protein family. Enzymes in this family require oxygen, ascorbic acid, 2-oxoglutaric acid, and Fe^{2+} for catalytic activity, and loss of any one of these factors reduces activity (45). Thus, PHD and FIH actively hydroxylate and thereby negatively regulate HIF under normoxic conditions when oxygen is abundant and are inhibited in hypoxia, enabling HIF to become active.

Three PHD proteins have been identified: PHD1, 2, and 3 (37). *In vitro* all three robustly hydroxylate HIF-1α (46). However, *in vivo* PHD2 plays a major role in normoxic HIF-α regulation, and it and PHD3 function in either mild or prolonged hypoxic conditions (47, 48). The role of PHD1 in HIF-1α regulation *in vivo* is not clear, although it reportedly hydroxylates IKK proteins (49). Recent reports also indicate that FIH hydroxylates and regulates the activity of Notch and IκB, both in the ankyrin repeat domains (50-52).

Several groups have generated PHD knockout mice. PHD2−/− mice showed embryonic lethality between E12.5 and 14.5, whereas PHD1 and 3−/− mice were apparently normal (53). PHD1 nulls exhibited altered anaerobic metabolism and lower oxygen consumption in skeletal muscle. This metabolic defect causes hypoxic tolerance in mice mainly because of impaired HIF-2α regulation (54). Conditional inactivation of PHD2 in mice increases EPO production, leading to polycythemia and congestive heart failure, possibly due to continuous expression of high levels of HIF (55). PHD3−/− mice show decreased rates of neuronal apoptosis, resulting in increased size of the superior cervical ganglion, adrenal medulla, and carotid body and overall impaired sympathoadrenal development (56). These animal models indicate an important role for PHDs in physiological normoxia and mild hypoxic conditions.

Siah2 and Hypoxia

The Ubiquitin System and Ubiquitin Ligase Siah2

Protein ubiquitination plays a critical role in the hypoxia response. For example, under hypoxia, the ubiquitination of HIF-1α and p53 is inhibited, whereas MyoD or IκB ubiquitination is promoted (57-59). Ubiquitination is achieved by sequential reaction of ubiquitin-activating enzyme E1, ubiquitin-transferring enzyme E2, and a substrate-specific ubiquitin ligase E3 (60). Substrates recognized by E3 are either mono- or poly-ubiquitinated and degraded, or in some cases, altered in terms of subcellular localization or protein-protein interaction (61). Siah2 is a RING finger type ubiquitin ligase with a catalytic RING domain on its N-terminus, followed by two zinc fingers and a C-terminal substrate binding domain (SBD; 62). Siah is a mammalian homolog of Sina, a *Drosophila* protein functioning in eye development (63, 64). Mammals express two isoforms, Siah1 and 2, and mice exhibit two Siah1 isotypes, 1a and 1b (65). A number of Siah substrates have been identified, including *N*-CoR, β-catenin, TRAF2, 2-oxoglutarate dehydrogenase-complex protein E2 (OGDC-E2), and TIEG (66-70; see Table 1 for detail). In some cases, interaction requires an adaptor protein such as phyllopod (PHYL) and Siah-interacting protein (SIP) to degrade *Drosophila* Tramtrack and mammalian β-catenin, respectively (see below; 67, 71). Siah2 null mice exhibit mild phenotypes, such as minor increases in the number of hematopoietic progenitor cells (72). However, Siah1a and Siah2 double nulls exhibit embryonic lethality, indicating an essential function in embryogenesis.

Table 1. Siah2 Substrates

Substrate category	Siah-Interacting Protein	Degradation	Known role	Reference No.
Transcriptional regulation	N-CoR	Yes	Degradation antagonizes the transcriptional inhibition by Nco-R	(96)
	c-Myb	Yes	Degradation is induced by p53 signal and downregulates HSP	(97)
	BOB-1/OBF1	Yes	Transcription in B cells	(98, 99)
	PML	Yes	Its degradation suppresses oncogenic form-mediated transcription	(100)
	CtIP	Yes	Interaction and degradation. Induces p21 independent of degradation	(101)
	TIEG-1	Yes	Regulation of transcription upon TGF β stimuli	(102)
Enzymes	α-ketoglutarate dehydrogenase	Yes	Enzyme involved in TCA cycle, increased activity in Siah null cells	(103)
	PHD	Yes	Regulation of HIF-1α expression through proline hydroxylation	(104)
	FIH	Yes	Regulation of HIF-1α transcriptional activity through asparagine hydroxylation	(105)
Cancers	β-catenin	Yes	Transcription factor mutated in multiple cancers	(106)
	DCC	Yes	Interaction and co-localization with Siah in the cells	(107)
Neuronal	AF4	Yes	Mutated in robotic mice, loss of its degradation increases transcription	(108, 109)
	α-synuclein	Yes/No	Interaction and monoubiquitinated by Siah2 *in vitro*	(110)
	Synphilin-1	Yes	Interaction with Siah and degradation, increases dopamine release	(110, 111)
	Synaptophysin	Yes	Co-localizes with Siah	(112)
	Group1 glutamate receptor	Yes	Long spliced form of mGluR1 is downregulated	(113)
Others	Peg3/Pw1	Yes	Functions cooperatively with Siah to induce cell death	(114)
	Kid	Yes	Its degradation alters mitosis	(115)
	Numb	Yes	Degradation of numb leads to increase in Notch signals	(116)
	T-STAR	Yes	Degradation modulates the alternative splicing	(117)
	Tramtrack	Yes	Transcriptional repressor involved in eye development of Drosophila	(118, 119)
	α-tubulin	No	Cytoskeletal protein	(115)
	pAPC	No	Included in the complex to degrade β-catenin	(106, 120)
	Ubc H5	No	E2 component of protein ubiquitination	(106)
	Vav	No	Rac1-GDP/GTP exchanger protein, its activity is inhibited by Siah	(121)
	BAG-1	No	Hsp70 binding protein involved in proliferation, death, and migration	(122)
	Dab-1	No	Interaction and inhibition of Siah activity	(123)
	Peg10	No	Interaction with Siah, overexpression of PEG10 suppresses apoptosis	(124)
	Phyllopod	No	Adaptor protein required for tramtrack degradation by Siah	(125)

Abbreviations: BOB-1, B cell Oct-binding protein 1; OBF1, Oct binding factor 1; PML, promyelocytic leukemia protein; CtIP, C-terminal binding protein (CtBP)-interacting protein; TIEG-1, TGFβ-inducible early gene-1; FIH, factor-inhibiting HIF; AF4, acute lymphoblastic leukemia 1-fused gene from chromosome 4; Peg, paternally expressed gene; Kid, kinesin-like DNA binding protein; T-STAR, testis-signal transduction and activation of RNA; BAG, Bcl-2-associated athanogene; Dab, disabled.

Role of Siah2/PHD Pathway in Hypoxia Response: from Cells to Animals

To search for proteins interacting with Siah2 we undertook mass spectrometric and co-immunoprecipitation analyses. These analyses identified PHDs 1, 2, and 3. Active degradation of PHD3 and a lesser degree of PHD1 were seen in 293T cells. Degradation was inhibited by treatment with the proteasome inhibitor MG132, supporting the idea that Siah2 targets PHD3 and PHD1 for degradation via the ubiquitin-proteasome pathway (73). Interestingly, although PHD2 was also found to associate with Siah2, PHD2 stability was not affected by the ligase. Among the mechanisms underlying selective targeting of PHD3 but not PHD2 by Siah2 is their distinct subcellular localization.[3]

Because PHD3 regulates HIF-1α stability through hydroxylation, the hypoxia response was monitored in mouse embryonic fibroblasts (MEF) established from either Siah2 knockout or wild type mice. HIF-1α levels were significantly reduced in MEFs derived from Siah2 nulls, and induction of a HIF-1α target, VEGF, was decreased in response to hypoxia. Importantly, MEFs derived from Siah1a/Siah2 double nulls exhibited more severe phenotypes and almost no HIF-1α expression or VEGF induction. These outcomes likely underlie the embryonic or neonatal lethality of Siah1a/2 double null mice (72). Because PHD3 is a primary Siah2 target and likely plays an important role in HIF-1α expression in some cell types, PHD3 expression was evaluated in Siah2 null MEFs relative to wild type controls. Importantly, inhibition of PHD3 expression by RNAi in Siah1a/2 double null MEFs rescued HIF-1α expression, suggesting that PHD3 mediates decreased HIF-1α expression in the absence of Siah2. Furthermore, a hypoxic environment enhanced the ability of Siah2 to degrade PHD3. The Siah2 mRNA levels increased in as little as 2 hours after cells were exposed to hypoxic conditions, an increase that was also seen under physiological normoxic to mild hypoxic conditions (5% O_2; Fig. 1, also see below).

In vivo experiments with Siah2 knockout mice exposed to hypoxic conditions (7% O_2) were undertaken to examine hemoglobin concentration and ventilatory responses. Wild type mice exposed to hypoxia exhibit a moderately higher, albeit statistically significant, increase in blood hemoglobin concentration within 14 days. In terms of the ventilatory response, wild type mice undergoing acute phase hypoxia (in 15 minutes) exhibited decreased metabolism and increased ventilation. By contrast, Siah2 null mice significantly decreased their metabolism but could not increase ventilation. Changes seen in the Siah2 knockout animals resemble those seen in the HIF-1α heterozygous animals (74) and are expected to be more pronounced in the absence of Siah1a and Siah1b genes.

Regulation of PHD3 Activity by Complex Formation

Gel filtration analyses indicate that a proportion of PHD3 exists as a protein complex in cultured cells (75). This complex exhibits a higher molecular weight upon hypoxia treatment, which is reversible when cells become reoxygenated. PHD3 protein in the complex exhibits decreased HIF hydroxylation

[3] K. Nakayama and Z. Ronai, unpublished data.

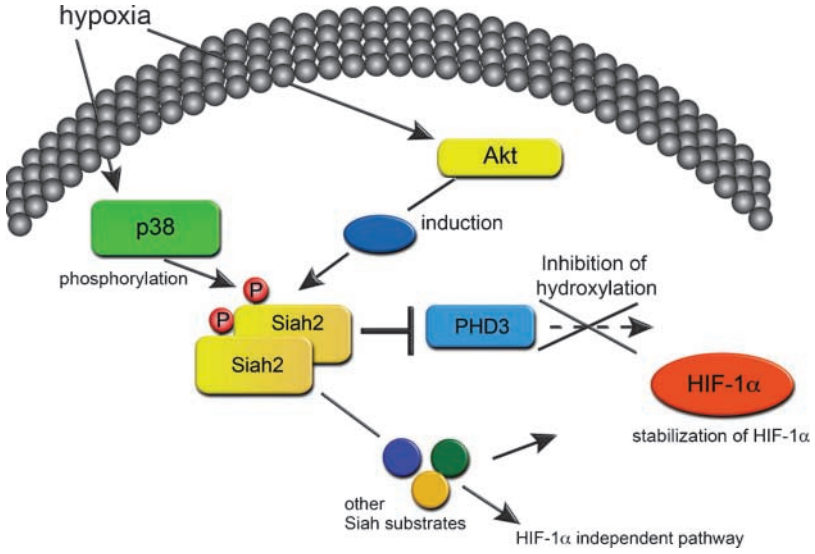

FIGURE 1. Hypoxic signaling connected to Siah. Under the hypoxia condition, hypoxia-activated p38 and Akt pathways positively regulate Siah2 activity through phosphorylation and induction, respectively. Siah2 is phosphorylated by p38 on its serine and threonine residues, which will enhance its activity. Akt pathway increases the abundance of Siah2 by induction of its mRNA through signaling pathways yet to be clarified. Induced and activated Siah2 modulates the hypoxic signaling through PHD3 degradation and HIF-1α stabilization on one hand. Although, it is not yet identified, other Siah2 substrates would also play roles on hypoxic signaling by both HIF-1α-dependent and -independent mechanism.

activity. Thus, complex formation may serve as a secondary mechanism to negatively regulate PHD3 activity such as in prolonged hypoxia. Siah2 was mostly found in a smaller molecular weight fraction, suggesting that PHD3 subject to active degradation by Siah2 does not reside in the large complex. Recently, PHD3 aggregates have been observed under normoxia condition by immunocytochemistry (76). The aggregate formation is dependent on the prolyl-hydroxylase activity of PHD3. These aggregates contain the proteasome component LMP-2 and appear to be scattered in the cytoplasm. The larger PHD3 complex and these aggregates likely represent two independent structures. However, the fact that PHD3 is found in both suggests that its activity is dynamically regulated by association and dissociation with specific complexes. The key question remains what would be the components of those structures.

Phosphorylation of Siah2 by p38

The hypoxia response involves multiple signaling cascades, including Akt or MAPK kinase pathways (77). Among those kinases, p38 MAPK is activated under hypoxic conditions in a manner partly dependent on reactive oxygen species (ROS) produced in mitochondria (78). p38 activity or that of the upstream kinases MKK3 or 6 is important to stabilize HIF-1α under hypoxia conditions, as shown in MEFs derived from various kinase knockout mice (79). Siah2 is subjected to phosphorylation, which increases its activity under hypoxia conditions (Fig. 1; 80). This phosphorylation of Siah2 was inhibited when cells were treated with p38 inhibitor SB203580. Siah2 contains the SQ/TQ amino acid sequence motif, a conserved motif for p38 phosphorylation. Phosphopeptide mapping or mutational analyses revealed S29 and T24 as major sites of Siah2 phosphorylation. Phosphomimetic mutants (T24E or S29D) showed gain-of-function activity and enhanced HIF-1α expression. Overall these studies indicate that phosphorylation-dependent Siah2 activation is sufficient to activate a component of the hypoxia response.

Induction of Siah2 mRNA Has a Possible Role for the Akt Pathway

Akt signaling functions to induce HIF-1α expression. First, growth factors, such as insulin-like growth factor (IGF-1), induce HIF-1α expression independent of hypoxia (81). Second, in some cell types, such as glioblastoma cells, hypoxia itself induces Akt activation and concomitant HIF-1α stabilization through an unknown mechanism (82). Siah2 mRNA induction by hypoxia (Fig. 1) is blocked by treatment with the PI3K inhibitor LY294002.[4] LY294002 treatment also decreases Akt activity. Conversely, Siah2 is induced when an active form of Akt is introduced into cells. Therefore, hypoxic activation of Akt would be one of the critical signals to induce Siah2 expression. Ongoing studies are addressing what transcription factors mediate Akt-dependent regulation of Siah2 transcription.

The Role of Siahs in Hypoxia and Tumorgenesis

Tumors generate local hypoxic regions, triggering a response necessary to recruit new vasculature required for tumor cell survival. Because of the key role played by HIF-1α in tumor development, we examined potential involvement of Siahs in tumorigenesis and metastasis using a syngeneic xenograft model of SW1 melanoma cells. SW1 melanoma cells were stably transfected with two types of Siah inhibitors (PHYL peptides or a dominant negative Siah2 RING mutant). Peptides derived from the PHYL sequence (PHYL peptides) bind to the Siah SBD with high affinity (83) and disrupt PHD3 binding, thereby elevating PHD3 levels and reducing HIF-1α levels (84). Whereas PHYL peptide expressing cells affect Siah2 targeting of substrates mediated by SBD, Siah2 RING mutant-expressing cells attenuate general function of Siah2 by serving as a dominant negative form. The two types of cells (PHYL and RING mutant expressing) were subcutaneously injected into C3H mice to monitor potential changes in tumor formation and lung metastases.

[4] K. Nakayama, J. Qi, and Z. Ronai, unpublished data.

Expression of PHYL peptide in SW1 cells ($SW1^{PHYL}$) used in xenograft models did not alter primary tumor size but rather inhibited lung metastasis. SW1 cells expressing IPAS, a potent inhibitor of HIF transcriptional activity (24), show similar phenotypes. Furthermore, ectopic re-expression of HIF-1α in $SW1^{PHYL}$ cells rescues the deficiency in lung metastasis with no change in primary tumor size, indicating overall that the Siah effect on metastasis is dependent on HIF-1α (Fig. 2). Although reduced HIF-1α staining compared to the parental SW1 cells is observed in both primary tumor cells and metastases formed by $SW1^{PHYL}$ cells, metastatic loci derived from $SW1^{PHYL}$ cells showed decreased vasculogenesis and proliferation concomitant with increased cell death. Therefore, Siah seems to promote vasculogenesis, cell growth, and survival in lung metastatic lesions, in part via its effect on HIF-1α levels. These observations indicate that the Siah2-PHD3 pathway plays an important role in tumor development, although this regulatory axis does not affect PHD2, which is considered to be the prominent PHD that regulates HIF-1α under normoxia condition. In addition, our results suggest that organ microenvironment plays a critical role in determining Siah and consequently HIF-1α effect on tumor development and progression. Specifically, conditional knockout of HIF-1α in the mammary epithelium of MMTV-PyMT mice significantly inhibits formation of lung metastases but has less effect on growth of the primary mammary tumor (85).

Unexpectedly, we found that expression of the dominant negative Siah2 RING mutant (Siah2-Rm) in SW1 melanoma cells ($SW1^{S2RM}$) resulted in reduced growth of primary tumors and decreased the number of lung metastases in the xenograft model (Fig. 2). Although Siah2-Rm alone has little effect on levels of PHD3 and HIF-1α in SW1 cells, it robustly upregulates levels of Sprouty2 (SPRY2), a specific inhibitor of Ras/ERK signaling. SPRY2 suppresses lung tumorigenesis in a K-ras transgenic mouse model (86) and has been identified as a Siah2 substrate (87). ShRNA knockdown of SPRY2 in $SW1^{Siah2\text{-}Rm}$ cells rescues ERK signaling deficiencies *in vitro* and tumorigenesis *in vivo*, indicating that Siah2 regulates tumorigenesis via SPRY2/ERK but independent of PHD3/HIF-1α (Fig. 2). Of note, although the possibility that Siah2-Rm affects tumorigenesis through its effect on other Siah2 substrates should be considered, the primary role of SPRY2 in mediating the change in tumorigenicity is confirmed by a shRNA experiment in which inhibition of SPRY2 abolishes the effect of Siah2-Rm.

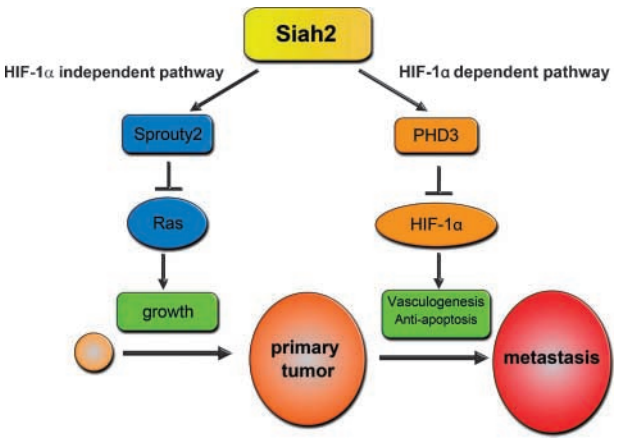

FIGURE 2. Regulation of HIF-dependent and -independent pathways by Siah2 on tumorigenesis and metastasis. Siah2 regulates the tumor growth stage by degradation of SPRY2 and subsequent activation of the Ras-ERK pathway, which is HIF-independent. Metastatic stage is also regulated by Siah2 through its effect on the PHD3-HIF pathway, which involves the growth or angiogenic ability of metastatic cells as well as maintaining their viability.

Importantly, initial analysis of a panel of human melanoma samples reveals an inverse correlation between levels of PHD3 and SPRY2 and the progression of malignancy, pointing to an oncogenic role of Siah2 in human melanomas.

Consistent with these findings, several groups report a role for Siah2 in tumorigenesis and metastasis of diverse tumor types. Siah2 activity is correlated with pancreatic tumor development likely through its positive effect on Ras signaling (88). This observation supports our studies of Siah2-SPRY2 in melanoma tumor development (84). Further, inhibiting Siah2 activity with peptides designed to interfere with Siah2 association with substrates efficiently inhibited mouse mammary tumor development and metastasis (89). Consistent with these data, crossing Siah2 null mice with the TRAMP prostate cancer mouse model resulted in marked inhibition of primary and metastatic tumors.[5] Furthermore increased expression of SIP is seen in pancreatic and nasopharyngeal tumors. Increased SIP levels likely enhance targeting of substrates requiring this adaptor protein (90). However, in renal cancer cells, SIP is downregulated and overexpression leads to growth inhibition (91). Clearly, the functional significance of adaptor proteins on tumorigenesis is complex and requires further investigation.

In summary, Siah ligases regulate tumorigenesis and metastasis by targeting parallel pathways mediated by SPRY2/Ras and PHD3/HIF, respectively (Fig. 2). The differential effect of PHYL peptide and Siah2-RM on PHD3 and SPRY2 seems to be due to substrate specificity of the Siah isoform (Siah1 versus Siah2) and to mechanisms used by Siah to bind substrates [via the SBD (adaptor is required for some substrates) versus SBD-independent]. Our data suggest that both Siah1 and Siah2 are required to target PHD3 via the Siah SBD and both may utilize an unknown adaptor, whereas Siah2 alone is sufficient to target SPRY2, possibly via direct binding through a region outside the SBD (Fig. 3).

Concluding Remarks

HIF-α proteins have been extensively studied because their expression is regulated in an oxygen-dependent manner and they regulate expression of numbers of genes enabling cellular adaptation to a hypoxia environment. However, it has also been suggested that (1) there are hypoxia responses independent of HIF, such as the mTOR pathway, and (2) that HIF may function in nonhypoxic situations following growth factor stimulation (81, 92). In addition to HIF, additional factors may be up- or downregulated following exposure to hypoxia. One potential means of regulating these factors could be through ubiquitin-dependent degradation, and Siah substrates are candidates for

[5] J. Qi, K. Nakayama, and Z. Ronai, unpublished data.

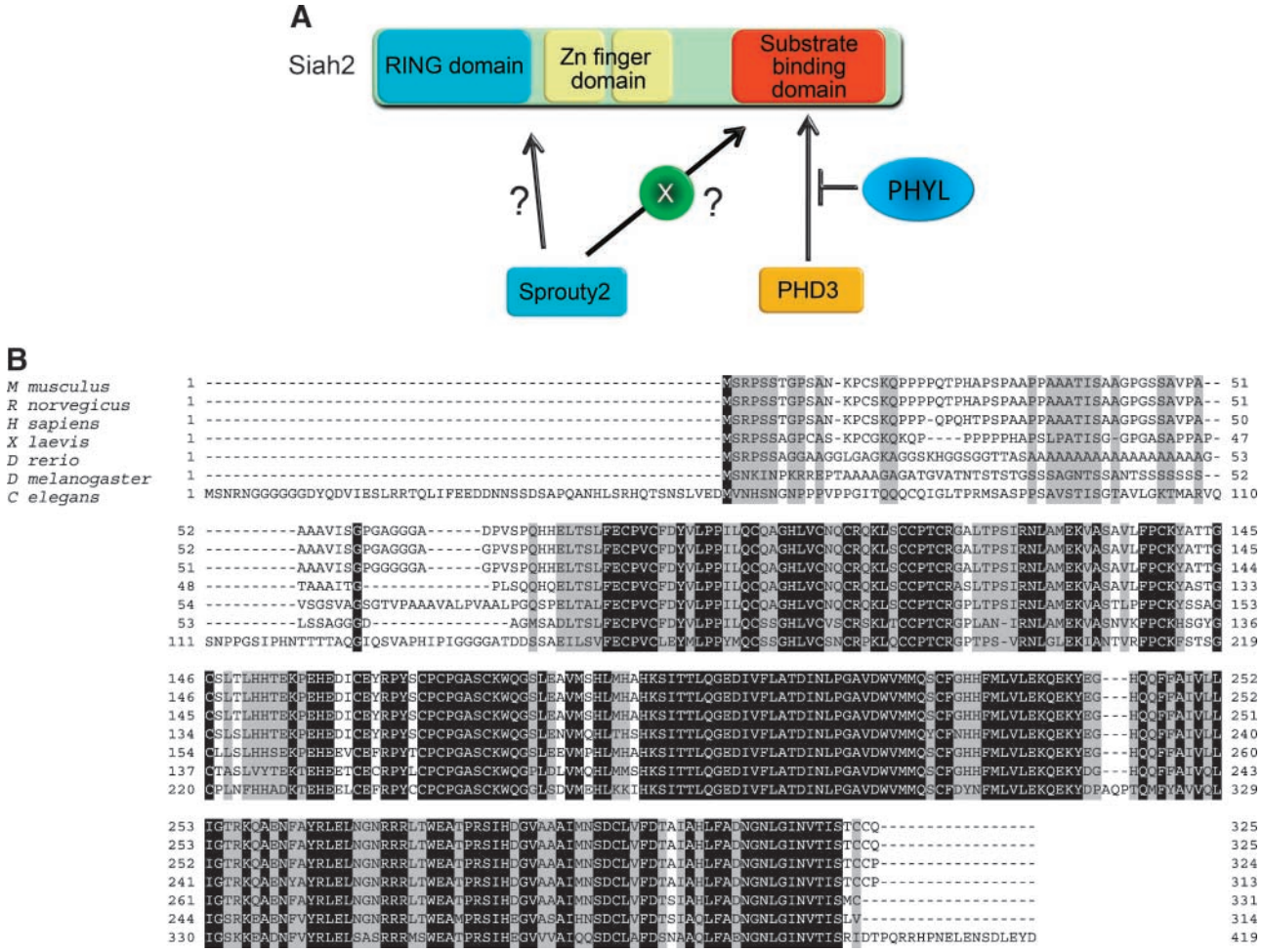

FIGURE 3. Interaction of Siah2 and Substrates. **A.** Siah2 substrates including PHD3 interact with SBD of Siah2, become ubiquitinated, and are subsequently degraded. PHYL peptide derived from adaptor protein phyllopod masks the SBD to interfere with the binding of the substrates, therefore serving as an inhibitor of Siah. SPRY2 is an exceptional case whose degradation is not affected by PHYL peptide, suggesting that it binds the region outside of SBD or uses some intermediate protein that has higher affinity to Siah2 than PHYL. These two different modes of interaction enable Siah2 to play different roles during tumorigenesis and metastasis. **B.** Alignment of Siah2 amino acid sequences among the species. *Mus musculus* (Siah2), *Rattus norvegicus* (Siah2), *Homo sapiens* (Siah2), *Xenopus laevis* (Siah2), *Danio rerio*(Siah2-like), *Drosophila melanogaster* (Sina), and *Caenorhabditis elegans* (Siah) sequences are compared. Completely identical amino acid residues throughout the species are boxed in black, and the residues with at least five matches out of seven are boxed in gray. Alignment was done by Clustal W program (http://clustalw.ddbj.nig.ac.jp/top-e.html).

this process. As Siah2 activity is increased by phosphorylation and induced under hypoxia, other factors may also be regulated similarly to PHD3. It is reported that OGDC-E2 is a Siah2 target (68). Although factors signaling OGDC-E2 degradation are not well understood, chronic hypoxia in rat brains decreases OGDC activity, and Siah2 may function in this process (93). Thus far, however, we have not detected changes in levels of two Siah substrates, TRAF2 and β-catenin, under hypoxic conditions,[4] raising the question of how specificity is achieved when several substrates are degraded by one E3 ligase. This could be explained by spatial expression patterns or localization of ligases and substrates. It is noteworthy that Siah substrate specificity seems to be dependent on the types of stresses cells encounter. For example, Siah1 promotes β-catenin degradation following UV-induced DNA damage, whereas Siah2 causes TRAF2 degradation upon TNF-α-induced apoptosis (67, 69). Several mechanisms likely underlie stress-dependent substrate specificity. First, different stimuli affect ligase expression. For example, Siah1 expression is induced by p53, whereas Siah2 expression is induced by hypoxia (73, 94). Second, different stimuli might alter subcellular localization of ligases or substrates. For example, acidosis induces nucleolar sequestration of pVHL to allow HIF-1α stabilization under normoxia (95). Third, different stimuli may alter posttranslational modification of ligases or substrates, which could in turn change the localization of proteins or the affinity of ligase/substrate interactions. For example, p38-induced phosphorylation of Siah2 can enhance its affinity to PHD3 (80).

Importantly, Siah proteins are found from human to fly and worm. Major functional domains of Siah2, N-terminus RING domain, Zinc-finger domain, and C-terminus SBD, are all highly conserved among the species (Fig. 3B). Thus, it is expected that Siah2 exerts its control of HIF-dependent and -independent pathways throughout the evolution of these species.

A key note in the recent studies is that Siah can promote tumorigenesis and metastasis by targeting SPRY2/Ras/ERK and PHD3/HIF-1α pathways. Because these pathways are critical in human cancers, the study highlights the potential to develop Siah inhibitors. Further investigations will clarify the role of Siah in cancer, such as whether there are any other substrates involved in the process, or a possibility of different mechanisms in different types of cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Lahiri S, Roy A, Baby SM, Hoshi T, Semenza GL, Prabhakar NR. Oxygen sensing in the body. Prog Biophys Mol Biol 2006;91:249–86, PubMed doi:10.1016/j.pbiomolbio.2005.07.001.

2. Bertout JA, Patel SA, Simon MC. The impact of O2 availability on human cancer. Nat Rev Cancer 2008;8:967–75, PubMed doi:10.1038/nrc2540.

3. Rankin EB, Giaccia AJ. The role of hypoxia-inducible factors in tumorigenesis. Cell Death Differ 2008;15:678–85, PubMed doi:10.1038/cdd.2008.21.

4. Wenger RH, Stiehl DP, Camenisch G. Integration of oxygen signaling at the consensus HRE. Sci STKE 2005;2005:re12.

5. Wykoff CC, Pugh CW, Maxwell PH, Harris AL, Ratcliffe PJ. Identification of novel hypoxia dependent and independent target genes of the von Hippel-Lindau (VHL) tumour suppressor by mRNA differential expression profiling. Oncogene 2000;19:6297–305, PubMed doi:10.1038/sj.onc.1204012.

6. Feldser D, Agani F, Iyer NV, Pak B, Ferreira G, Semenza GL. Reciprocal positive regulation of hypoxia-inducible factor 1α and insulin-like growth factor 2. Cancer Res 1999;59:3915–8, PubMed.

7. Krishnamachary B, Berg-Dixon S, Kelly B, et al. Regulation of colon carcinoma cell invasion by hypoxia-inducible factor 1. Cancer Res 2003;63:1138–43, PubMed.

8. Bruick RK. Expression of the gene encoding the proapoptotic Nip3 protein is induced by hypoxia. Proc Natl Acad Sci U S A 2000;97:9082–7, PubMed doi:10.1073/pnas.97.16.9082.

9. Sowter HM, Ratcliffe PJ, Watson P, Greenberg AH, Harris AL. HIF-1-dependent regulation of hypoxic induction of the cell death factors BNIP3 and NIX in human tumors. Cancer Res 2001;61:6669–73, PubMed.

10. Ebert BL, Firth JD, Ratcliffe PJ. Hypoxia and mitochondrial inhibitors regulate expression of glucose transporter-1 via distinct Cis-acting sequences. J Biol Chem 1995;270:29083–9, PubMed doi:10.1074/jbc.270.36.21021.

11. Firth JD, Ebert BL, Pugh CW, Ratcliffe PJ. Oxygen-regulated control elements in the phosphoglycerate kinase 1 and lactate dehydrogenase A genes: similarities with the erythropoietin 3′ enhancer. Proc Natl Acad Sci U S A 1994;91:6496–500, PubMed doi:10.1073/pnas.91.14.6496.

12. Semenza GL, Roth PH, Fang HM, Wang GL. Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. J Biol Chem 1994;269:23757–63, PubMed.

13. Graven KK, Yu Q, Pan D, Roncarati JS, Farber HW. Identification of an oxygen responsive enhancer element in the glyceraldehyde-3-phosphate dehydrogenase gene. Biochim Biophys Acta 1999;1447:208–18.

14. Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab 2006;3:177–85, PubMed doi:10.1016/j.cmet.2006.02.002.

15. Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. Cell Metab 2006;3:187–97, PubMed doi:10.1016/j.cmet.2006.01.012.

16. Forsythe JA, Jiang BH, Iyer NV, et al. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol Cell Biol 1996;16:4604–13.

17. Levy AP, Levy NS, Wegner S, Goldberg MA. Transcriptional regulation of the rat vascular endothelial growth factor gene by hypoxia. J Biol Chem 1995;270:13333–40, PubMed doi:10.1074/jbc.270.22.13333.

18. Liu Y, Cox SR, Morita T, Kourembanas S. Hypoxia regulates vascular endothelial growth factor gene expression in endothelial cells. Identification of a 5′ enhancer. Circ Res 1995;77:638–43.

19. Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol 1992;12:5447–54, PubMed.

20. Pennacchietti S, Michieli P, Galluzzo M, Mazzone M, Giordano S, Comoglio PM. Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. Cancer Cell 2003;3:347–61, PubMed doi:10.1016/S1535-6108(03)00085-0.

21. Staller P, Sulitkova J, Lisztwan J, Moch H, Oakeley EJ, Krek W. Chemokine receptor CXCR4 downregulated by von Hippel-Lindau tumour suppressor pVHL. Nature 2003;425:307–11, PubMed doi:10.1038/nature01874.

22. Gordan JD, Bertout JA, Hu CJ, Diehl JA, Simon MC. HIF-2α promotes hypoxic cell proliferation by enhancing c-myc transcriptional activity. Cancer Cell 2007;11:335–47, PubMed doi:10.1016/j.ccr.2007.02.006.

23. Holmquist-Mengelbier L, Fredlund E, Lofstedt T, et al. Recruitment of HIF-1α and HIF-2α to common target genes is differentially regulated in neuroblastoma: HIF-2α promotes an aggressive phenotype. Cancer Cell 2006;10:413–23, PubMed doi:10.1016/j.ccr.2006.08.026.

24. Makino Y, Cao R, Svensson K, et al. Inhibitory PAS domain protein is a negative regulator of hypoxia-inducible gene expression. Nature 2001;414:550–4, PubMed doi:10.1038/35107085.

25. Covello KL, Kehler J, Yu H, et al. HIF-2α regulates Oct-4: effects of hypoxia on stem cell function, embryonic development, and tumor growth. Genes Dev 2006;20:557–70, PubMed doi:10.1101/gad.1399906.

26. Iyer NV, Kotch LE, Agani F, et al. Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 α. Genes Dev 1998;12:149–62, PubMed doi:10.1101/gad.12.2.149.

27. Carmeliet P, Dor Y, Herbert JM, et al. Role of HIF-1α in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. Nature 1998;394:485–90, PubMed doi:10.1038/28867.

28. Ryan HE, Lo J, Johnson RS. HIF-1 α is required for solid tumor formation and embryonic vascularization. EMBO J 1998;17:3005–15, PubMed doi:10.1093/emboj/17.11.3005.

29. Compernolle V, Brusselmans K, Acker T, et al. Loss of HIF-2α and inhibition of VEGF impair fetal lung maturation, whereas treatment with VEGF prevents fatal respiratory distress in premature mice. Nat Med 2002;8:702–10, PubMed.

30. Peng J, Zhang L, Drysdale L, Fong GH. The transcription factor EPAS-1/hypoxia-inducible factor 2α plays an important role in vascular remodeling. Proc Natl Acad Sci U S A 2000;97:8386–91, PubMed doi:10.1073/pnas.140087397.

31. Scortegagna M, Ding K, Oktay Y, et al. Multiple organ pathology, metabolic abnormalities and impaired homeostasis of reactive oxygen species in Epas1−/− mice. Nat Genet 2003;35:331–40, PubMed doi:10.1038/ng1266.

32. Tian H, Hammer RE, Matsumoto AM, Russell DW, McKnight SL. The hypoxia-responsive transcription factor EPAS1 is essential for catecholamine homeostasis and protection against heart failure during embryonic development. Genes Dev 1998;12:3320–4, PubMed doi:10.1101/gad.12.21.3320.

33. Kamura T, Sato S, Iwai K, Czyzyk-Krzeska M, Conaway RC, Conaway JW. Activation of HIF1α ubiquitination by a reconstituted von Hippel-Lindau (VHL) tumor suppressor complex. Proc Natl Acad Sci U S A 2000;97:10430–5, PubMed doi:10.1073/pnas.190332597.

34. Ohh M, Park CW, Ivan M, et al. Ubiquitination of hypoxia-inducible factor requires direct binding to the β-domain of the von Hippel-Lindau protein. Nat Cell Biol 2000;2:423–7, PubMed doi:10.1038/35017054.

35. Maxwell PH, Wiesener MS, Chang GW, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 1999;399:271–5, PubMed doi:10.1038/20459.

36. Ivan M, Kondo K, Yang H, et al. HIFα targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. Science 2001;292:464–8, PubMed doi:10.1126/science.1059817.

37. Epstein AC, Gleadle JM, McNeill LA, et al. C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. Cell 2001;107:43–54, PubMed doi:10.1016/S0092-8674(01)00507-4.

38. Hon WC, Wilson MI, Harlos K, et al. Structural basis for the recognition of hydroxyproline in HIF-1 α by pVHL. Nature 2002;417:975–8, PubMed doi:10.1038/nature00767.

39. Min JH, Yang H, Ivan M, Gertler F, Kaelin WG, Jr., Pavletich NP. Structure of an HIF-1α -pVHL complex: hydroxyproline recognition in signaling. Science 2002;296:1886–9, PubMed doi:10.1126/science.1073440.

40. Richard DE, Berra E, Gothie E, Roux D, Pouyssegur J. p42/p44 mitogen-activated protein kinases phosphorylate hypoxia-inducible factor 1α (HIF-1α)

and enhance the transcriptional activity of HIF-1. J Biol Chem 1999;274:32631–7, PubMed doi:10.1074/jbc.274.46.32631.

41. Carbia-Nagashima A, Gerez J, Perez-Castro C, et al. RSUME, a small RWD-containing protein, enhances SUMO conjugation and stabilizes HIF-1α during hypoxia. Cell 2007;131:309–23, PubMed doi:10.1016/j.cell.2007.07.044.

42. Cheng J, Kang X, Zhang S, Yeh ET. SUMO-specific protease 1 is essential for stabilization of HIF1α during hypoxia. Cell 2007;131:584–95, PubMed doi:10.1016/j.cell.2007.08.045.

43. Bae SH, Jeong JW, Park JA, et al. Sumoylation increases HIF-1α stability and its transcriptional activity. Biochem Biophys Res Commun 2004;324:394–400, PubMed doi:10.1016/j.bbrc.2004.09.068.

44. Lando D, Peet DJ, Gorman JJ, Whelan DA, Whitelaw ML, Bruick RK. FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. Genes Dev 2002;16:1466–71, PubMed doi:10.1101/gad.991402.

45. Schofield CJ, Ratcliffe PJ. Signalling hypoxia by HIF hydroxylases. Biochem Biophys Res Commun 2005;338:617–26, PubMed doi:10.1016/j.bbrc.2005.08.111.

46. Bruick RK, McKnight SL. A conserved family of prolyl-4-hydroxylases that modify HIF. Science 2001;294:1337–40, PubMed doi:10.1126/science.1066373.

47. Berra E, Benizri E, Ginouves A, Volmat V, Roux D, Pouyssegur J. HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1α in normoxia. EMBO J 2003;22:4082–90, PubMed doi:10.1093/emboj/cdg392.

48. Appelhoff RJ, Tian YM, Raval RR, et al. Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor. J Biol Chem 2004;279:38458–65, PubMed doi:10.1074/jbc.M406026200.

49. Cummins EP, Berra E, Comerford KM, et al. Prolyl hydroxylase-1 negatively regulates IkappaB kinase-β, giving insight into hypoxia-induced NFkappaB activity. Proc Natl Acad Sci U S A 2006;103:18154–9, PubMed doi:10.1073/pnas.0602235103.

50. Cockman ME, Lancaster DE, Stolze IP, et al. Posttranslational hydroxylation of ankyrin repeats in IkappaB proteins by the hypoxia-inducible factor (HIF) asparaginyl hydroxylase, factor inhibiting HIF (FIH). Proc Natl Acad Sci U S A 2006;103:14767–72, PubMed doi:10.1073/pnas.0606877103.

51. Coleman ML, McDonough MA, Hewitson KS, et al. Asparaginyl hydroxylation of the Notch ankyrin repeat domain by factor inhibiting hypoxia-inducible factor. J Biol Chem 2007;282:24027–38, PubMed doi:10.1074/jbc.M704102200.

52. Zheng X, Linke S, Dias JM, et al. Interaction with factor inhibiting HIF-1 defines an additional mode of cross-coupling between the Notch and hypoxia signaling pathways. Proc Natl Acad Sci U S A 2008;105:3368–73, PubMed doi:10.1073/pnas.0711591105.

53. Takeda K, Ho VC, Takeda H, Duan LJ, Nagy A, Fong GH. Placental but not heart defects are associated with elevated hypoxia-inducible factor α levels in mice lacking prolyl hydroxylase domain protein 2. Mol Cell Biol 2006;26:8336–46, PubMed doi:10.1128/MCB.00425-06.

54. Aragones J, Schneider M, Van Geyte K, et al. Deficiency or inhibition of oxygen sensor Phd1 induces hypoxia tolerance by reprogramming basal metabolism. Nat Genet 2008;40:170–80, PubMed doi:10.1038/ng.2007.62.

55. Minamishima YA, Moslehi J, Bardeesy N, Cullen D, Bronson RT, Kaelin WG, Jr. Somatic inactivation of the PHD2 prolyl hydroxylase causes polycythemia and congestive heart failure. Blood 2008;111:3236–44, PubMed doi:10.1182/blood-2007-10-117812.

56. Bishop T, Gallagher D, Pascual A, et al. Abnormal sympathoadrenal development and systemic hypotension in PHD3−/− mice. Mol Cell Biol 2008;28:3386–400, PubMed doi:10.1128/MCB.02041-07.

57. Alarcon R, Koumenis C, Geyer RK, Maki CG, Giaccia AJ. Hypoxia induces p53 accumulation through MDM2 down-regulation and inhibition of E6-mediated degradation. Cancer Res 1999;59:6046–51, PubMed.

58. Di Carlo A, De Mori R, Martelli F, Pompilio G, Capogrossi MC, Germani A. Hypoxia inhibits myogenic differentiation through accelerated MyoD degradation. J Biol Chem 2004;279:16332–8, PubMed doi:10.1074/jbc.M313931200.

59. Taylor CT, Furuta GT, Synnestvedt K, Colgan SP. Phosphorylation-dependent targeting of cAMP response element binding protein to the ubiquitin/proteasome pathway in hypoxia. Proc Natl Acad Sci U S A 2000;97:12091–6, PubMed doi:10.1073/pnas.220211797.

60. Hershko A, Ciechanover A. The ubiquitin system. Annu Rev Biochem 1998;67:425–79, PubMed doi:10.1146/annurev.biochem.67.1.425.

61. Schnell JD, Hicke L. Non-traditional functions of ubiquitin and ubiquitin-binding proteins. J Biol Chem 2003;278:35857–60, PubMed doi:10.1074/jbc.R300018200.

62. Reed JC, Ely KR. Degrading liaisons: Siah structure revealed. Nat Struct Biol 2002;9:8–10, PubMed doi:10.1038/nsb0102-8.

63. Genbacev O, Zhou Y, Ludlow JW, Fisher SJ. Regulation of human placental development by oxygen tension. Science 1997;277:1669–72, PubMed doi:10.1126/science.277.5332.1669.

64. Tang AH, Neufeld TP, Kwan E, Rubin GM. PHYL acts to down-regulate TTK88, a transcriptional repressor of neuronal cell fates, by a SINA-dependent mechanism. Cell 1997;90:459–67, PubMed doi:10.1016/S0092-8674(00)80506-1.

65. Della NG, Senior PV, Bowtell DD. Isolation and characterisation of murine homologues of the Drosophila seven in absentia gene (sina). Development 1993;117:1333–43, PubMed.

66. Zhang J, Guenther MG, Carthew RW, Lazar MA. Proteasomal regulation of nuclear receptor corepressor-mediated repression. Genes Dev 1998;12:1775–80, PubMed doi:10.1101/gad.12.12.1775.

67. Matsuzawa SI, Reed JC. Siah-1, SIP, and Ebi collaborate in a novel pathway for β-catenin degradation linked to p53 responses. Mol Cell 2001;7:915–26, PubMed doi:10.1016/S1097-2765([01])00242-8.

68. Habelhah H, Laine A, Erdjument-Bromage H, et al. Regulation of 2-oxoglutarate (α-ketoglutarate) dehydrogenase stability by the RING finger ubiquitin ligase Siah. J Biol Chem 2004;279:53782–8, PubMed doi:10.1074/jbc.M410315200.

69. Habelhah H, Frew IJ, Laine A, et al. Stress-induced decrease in TRAF2 stability is mediated by Siah2. EMBO J 2002;21:5756–65, PubMed doi:10.1093/emboj/cdf576.

70. Johnsen SA, Subramaniam M, Monroe DG, Janknecht R, Spelsberg TC. Modulation of transforming growth factor β (TGFβ)/Smad transcriptional responses through targeted degradation of TGFβ-inducible early gene-1 by human seven in absentia homologue. J Biol Chem 2002;277:30754–9, PubMed doi:10.1074/jbc.M204812200.

71. Li S, Xu C, Carthew RW. Phyllopod acts as an adaptor protein to link the sina ubiquitin ligase to the substrate protein tramtrack. Mol Cell Biol 2002;22:6854–65, PubMed doi:10.1128/MCB.22.19.6854-6865.2002.

72. Frew IJ, Hammond VE, Dickins RA, et al. Generation and analysis of Siah2 mutant mice. Mol Cell Biol 2003;23:9150–61, PubMed doi:10.1128/MCB.23.24.9150-9161.2003.

73. Nakayama K, Frew IJ, Hagensen M, et al. Siah2 regulates stability of prolyl-hydroxylases, controls HIF1α abundance, and modulates physiological responses to hypoxia. Cell 2004;117:941–52, PubMed doi:10.1016/j.cell.2004.06.001.

74. Yu AY, Shimoda LA, Iyer NV, et al. Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1α. J Clin Invest 1999;103:691–6, PubMed doi:10.1172/JCI5912.

75. Nakayama K, Gazdoiu S, Abraham R, Pan ZQ, Ronai Z. Hypoxia-induced assembly of prolyl hydroxylase PHD3 into complexes: implications for its activity and susceptibility for degradation by the E3 ligase Siah2. Biochem J 2007;401:217–26, PubMed doi:10.1042/BJ20061135.

76. Rantanen K, Pursiheimo J, Hogel H, Himanen V, Metzen E, Jaakkola PM. Prolyl hydroxylase PHD3 activates oxygen-dependent protein aggregation. Mol Biol Cell 2008;19:2231–40, PubMed doi:10.1091/mbc.E07-11-1124.

77. Seta KA, Spicer Z, Yuan Y, Lu G, Millhorn DE. Responding to hypoxia: lessons from a model cell line. Sci STKE 2002;2002:RE11.

78. Kulisz A, Chen N, Chandel NS, Shao Z, Schumacker PT. Mitochondrial ROS initiate phosphorylation of p38 MAP kinase during hypoxia in cardiomyocytes. Am J Physiol Lung Cell Mol Physiol 2002;282:L1324–9, PubMed.

79. Emerling BM, Platanias LC, Black E, Nebreda AR, Davis RJ, Chandel NS. Mitochondrial reactive oxygen species activation of p38 mitogen-activated protein kinase is required for hypoxia signaling. Mol Cell Biol 2005;25:4853–62, PubMed doi:10.1128/MCB.25.12.4853-4862.2005.

80. Khurana A, Nakayama K, Williams S, Davis RJ, Mustelin T, Ronai Z. Regulation of the ring finger E3 ligase Siah2 by p38 MAPK. J Biol Chem 2006;281:35316–26, PubMed doi:10.1074/jbc.M606568200.

81. Fukuda R, Hirota K, Fan F, Jung YD, Ellis LM, Semenza GL. Insulin-like growth factor 1 induces hypoxia-inducible factor 1-mediated vascular endothelial growth factor expression, which is dependent on MAP kinase and phosphatidylinositol 3-kinase signaling in colon cancer cells. J Biol Chem 2002;277:38205–11, PubMed doi:10.1074/jbc.M203781200.

82. Zundel W, Schindler C, Haas-Kogan D, et al. Loss of PTEN facilitates HIF-1-mediated gene expression. Genes Dev 2000;14:391–6.

83. House CM, Hancock NC, Moller A, et al. Elucidation of the substrate binding site of Siah ubiquitin ligase. Structure 2006;14:695–701, PubMed doi:10.1016/j.str.2005.12.013.

84. Qi J, Nakayama K, Gaitonde S, et al. The ubiquitin ligase Siah2 regulates tumorigenesis and metastasis by HIF-dependent and -independent pathways. Proc Natl Acad Sci U S A 2008;105:16713–8, PubMed doi:10.1073/pnas.0804063105.

85. Liao D, Corle C, Seagroves TN, Johnson RS. Hypoxia-inducible factor-1α is a key regulator of metastasis in a transgenic model of cancer initiation and progression. Cancer Res 2007;67:563–72, PubMed doi:10.1158/0008-5472.CAN-06-2701.

86. Shaw AT, Meissner A, Dowdle JA, et al. Sprouty-2 regulates oncogenic K-ras in lung development and tumorigenesis. Genes Dev 2007;21:694–707, PubMed doi:10.1101/gad.1526207.

87. Nadeau RJ, Toher JL, Yang X, Kovalenko D, Friesel R. Regulation of Sprouty2 stability by mammalian Seven-in-Absentia homolog 2. J Cell Biochem 2007;100:151–60, PubMed doi:10.1002/jcb.21040.

88. Schmidt RL, Park CH, Ahmed AU, et al. Inhibition of RAS-mediated transformation and tumorigenesis by targeting the downstream E3 ubiquitin ligase seven in absentia homologue. Cancer Res 2007;67:11798–810, PubMed doi:10.1158/0008-5472.CAN-06-4471.

89. Möller A, House CM, Wong CS, et al. Inhibition of Siah ubiquitin ligase function. Oncogene 2008;28:289–96.

90. Zhai H, Shi Y, Jin H, et al. Expression of calcyclin-binding protein/Siah-1 interacting protein in normal and malignant human tissues: an immunohistochemical survey. J Histochem Cytochem 2008;56:765–72, PubMed doi:10.1369/jhc.2008.950519.

91. Sun S, Ning X, Liu J, et al. Overexpressed CacyBP/SIP leads to the suppression of growth in renal cell carcinoma. Biochem Biophys Res Commun 2007;356:864–71, PubMed doi:10.1016/j.bbrc.2007.03.080.

92. Arsham AM, Howell JJ, Simon MC. A novel hypoxia-inducible factor-independent hypoxic response regulating mammalian target of rapamycin and its targets. J Biol Chem 2003;278:29655–60, PubMed doi:10.1074/jbc.M212770200.

93. Lai JC, White BK, Buerstatte CR, Haddad GG, Novotny EJ, Jr., Behar KL. Chronic hypoxia in development selectively alters the activities of key enzymes of glucose oxidative metabolism in brain regions. Neurochem Res 2003;28:933–40, PubMed doi:10.1023/A:1023235712524.

94. Matsuzawa S, Takayama S, Froesch BA, Zapata JM, Reed JC. p53-inducible human homologue of Drosophila seven in absentia (Siah) inhibits cell growth: suppression by BAG-1. EMBO J 1998;17:2736–47, PubMed doi:10.1093/emboj/17.10.2736.

95. Mekhail K, Khacho M, Carrigan A, Hache RR, Gunaratnam L, Lee S. Regulation of ubiquitin ligase dynamics by the nucleolus. J Cell Biol 2005;170:733–44, PubMed doi:10.1083/jcb.200506030.

96. Zhang J, Guenther MG, Carthew RW, Lazar MA. Proteasomal regulation of nuclear receptor corepressor-mediated repression. Genes Dev 1998;12:1775–80, PubMed doi:10.1101/gad.12.12.1775.

97. Tanikawa J, Ichikawa-Iwata E, Kanei-Ishii C, et al. p53 suppresses the c-Myb-induced activation of heat shock transcription factor 3. J Biol Chem 2000;275:15578–85, PubMed doi:10.1074/jbc.M000372200.

98. Boehm J, He Y, Greiner A, Staudt L, Wirth T. Regulation of BOB.1/OBF.1 stability by SIAH. EMBO J 2001;20:4153–62, PubMed doi:10.1093/emboj/20.15.4153.

99. Tiedt R, Bartholdy BA, Matthias G, Newell JW, Matthias P. The RING finger protein Siah-1 regulates the level of the transcriptional coactivator OBF-1. EMBO J 2001;20:4143–52, PubMed doi:10.1093/emboj/20.15.4143.

100. Fanelli M, Fantozzi A, De Luca P, et al. The coiled-coil domain is the structural determinant for mammalian homologues of Drosophila Sina-mediated degradation of promyelocytic leukemia protein and other tripartite motif proteins by the proteasome. J Biol Chem 2004;279:5374–9, PubMed doi:10.1074/jbc.M306407200.

101. Germani A, Prabel A, Mourah S, et al. SIAH-1 interacts with CtIP and promotes its degradation by the proteasome pathway. Oncogene 2003;22:8845–51, PubMed doi:10.1038/sj.onc.1206994.

102. Johnsen SA, Subramaniam M, Monroe DG, Janknecht R, Spelsberg TC. Modulation of transforming growth factor β (TGFβ)/Smad transcriptional responses through targeted degradation of TGFβ-inducible early gene-1 by human seven in absentia homologue. J Biol Chem 2002;277:30754–9, PubMed doi:10.1074/jbc.M204812200.

103. Habelhah H, Laine A, Erdjument-Bromage H, et al. Regulation of 2-oxoglutarate (α-ketoglutarate) dehydrogenase stability by the RING finger ubiquitin ligase Siah. J Biol Chem 2004;279:53782–8, PubMed doi:10.1074/jbc.M410315200.

104. Nakayama K, Frew IJ, Hagensen M, et al. Siah2 regulates stability of prolyl-hydroxylases, controls HIF1α abundance, and modulates physiological responses to hypoxia. Cell 2004;117:941–52, PubMed doi:10.1016/j.cell.2004.06.001.

105. Fukuba H, Takahashi T, Jin HG, Kohriyama T, Matsumoto M. Abundance of aspargynyl-hydroxylase FIH is regulated by Siah-1 under normoxic conditions. Neurosci Lett 2008;433:209–14, PubMed doi:10.1016/j.neulet.2007.12.069.

106. Matsuzawa SI, Reed JC. Siah-1, SIP, and Ebi collaborate in a novel pathway for β-catenin degradation linked to p53 responses. Mol Cell 2001;7:915–26, PubMed doi:10.1016/S1097-2765(01)00242-8.

107. Hu G, Zhang S, Vidal M, Baer JL, Xu T, Fearon ER. Mammalian homologs of seven in absentia regulate DCC via the ubiquitin-proteasome pathway. Genes Dev 1997;11:2701–14, PubMed doi:10.1101/gad.11.20.2701.

108. Bursen A, Moritz S, Gaussmann A, Dingermann T, Marschalek R. Interaction of AF4 wild-type and AF4.MLL fusion protein with SIAH proteins: indication for t(4;11) pathobiology?. Oncogene 2004;23:6237–49, PubMed doi:10.1038/sj.onc.1207837.

109. Oliver PL, Bitoun E, Clark J, Jones EL, Davies KE. Mediation of Af4 protein function in the cerebellum by Siah proteins. Proc Natl Acad Sci U S A 2004;101:14901–6, PubMed doi:10.1073/pnas.0406196101.

110. Liani E, Eyal A, Avraham E, et al. Ubiquitylation of synphilin-1 and α-synuclein by SIAH and its presence in cellular inclusions and Lewy bodies imply a role in Parkinson's disease. Proc Natl Acad Sci U S A 2004;101:5500–5, PubMed doi:10.1073/pnas.0401081101.

111. Nagano Y, Yamashita H, Takahashi T, et al. Siah-1 facilitates ubiquitination and degradation of synphilin-1. J Biol Chem 2003;278:51504–14, PubMed doi:10.1074/jbc.M306347200.

112. Wheeler TC, Chin LS, Li Y, Roudabush FL, Li L. Regulation of synaptophysin degradation by mammalian homologues of seven in absentia. J Biol Chem 2002;277:10273–82, PubMed doi:10.1074/jbc.M107857200.

113. Moriyoshi K, Iijima K, Fujii H, Ito H, Cho Y, Nakanishi S. Seven in absentia homolog 1A mediates ubiquitination and degradation of group 1 metabotropic glutamate receptors. Proc Natl Acad Sci U S A 2004;101:8614–9, PubMed doi:10.1073/pnas.0403042101.

114. Relaix F, Wei X, Li W, et al. Pw1/Peg3 is a potential cell death mediator and cooperates with Siah1a in p53-mediated apoptosis. Proc Natl Acad Sci U S A 2000;97:2105–10, PubMed doi:10.1073/pnas.040378897.

115. Germani A, Bruzzoni-Giovanelli H, Fellous A, Gisselbrecht S, Varin-Blank N, Calvo F. SIAH-1 interacts with α-tubulin and degrades the kinesin Kid by the proteasome pathway during mitosis. Oncogene 2000;19:5997–6006, PubMed doi:10.1038/sj.onc.1204002.

116. Susini L, Passer BJ, Amzallag-Elbaz N, et al. Siah-1 binds and regulates the function of Numb. Proc Natl Acad Sci U S A 2001;98:15067–72, PubMed doi:10.1073/pnas.261571998.

117. Venables JP, Dalgliesh C, Paronetto MP, et al. SIAH1 targets the alternative splicing factor T-STAR for degradation by the proteasome. Hum Mol Genet 2004;13:1525–34, PubMed doi:10.1093/hmg/ddh165.

118. Li S, Li Y, Carthew RW, Lai ZC. Photoreceptor cell differentiation requires regulated proteolysis of the transcriptional repressor Tramtrack. Cell 1997;90:469–78, PubMed doi:10.1016/S0092-8674(00)80507-3.

119. Tang AH, Neufeld TP, Kwan E, Rubin GM. PHYL acts to down-regulate TTK88, a transcriptional repressor of neuronal cell fates, by a SINA-dependent mechanism. Cell 1997;90:459–67, PubMed doi:10.1016/S0092-8674(00)80506-1.

120. Liu J, Stevens J, Rote CA, et al. Siah-1 mediates a novel β-catenin degradation pathway linking p53 to the adenomatous polyposis coli protein. Mol Cell 2001;7:927–36, PubMed doi:10.1016/S1097-2765(01)00241-6.

121. Germani A, Romero F, Houlard M, et al. hSiah2 is a new Vav binding protein which inhibits Vav-mediated signaling pathways. Mol Cell Biol 1999;19:3798–807, PubMed.

122. Matsuzawa S, Takayama S, Froesch BA, Zapata JM, Reed JC. p53-inducible human homologue of Drosophila seven in absentia (Siah) inhibits cell growth: suppression by BAG-1. EMBO J 1998;17:2736–47, PubMed doi:10.1093/emboj/17.10.2736.

123. Park TJ, Hamanaka H, Ohshima T, Watanabe N, Mikoshiba K, Nukina N. Inhibition of ubiquitin ligase Siah-1A by disabled-1. Biochem Biophys Res Commun 2003;302:671–8, PubMed doi:10.1016/S0006-291X(03)00247-X.

124. Okabe H, Satoh S, Furukawa Y, et al. Involvement of PEG10 in human hepatocellular carcinogenesis through interaction with SIAH1. Cancer Res 2003;63:3043–8.

125. Li S, Xu C, Carthew RW. Phyllopod acts as an adaptor protein to link the sina ubiquitin ligase to the substrate protein tramtrack. Mol Cell Biol 2002;22:6854–65, PubMed doi:10.1128/MCB.22.19.6854-6865.2002.

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