Siah Proteins: Novel Drug Targets in the Ras and Hypoxia Pathways

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Abstract

The Siah (seven in absentia homolog) family of RING-domain proteins are components of ubiquitin ligase complexes, targeting proteins for proteasomal degradation. Siah family members have been reported to function in Ras, estrogen, DNA-damage, and hypoxia response pathways. Although earlier reports implicated Siah proteins as tumor suppressors, recent studies in mouse models have shown that Siah inhibition impairs tumor growth and metastasis. Given their central role in oncogenic and angiogenic pathways, Siah proteins are attractive novel therapeutic targets in cancer. [Cancer Res 2009;69(23):8835–8]

A Small Family of Highly Homologous E3 Ubiquitin Ligases

Experiments two decades ago described the first member of the family, seven in absentia (sina), as a protein required for formation of the R7 photoreceptor in *Drosophila* (1). Subsequent genetic and biochemical experiments showed that sina targeted a transcriptional repressor, tramtrack, for proteolytic degradation, allowing a key cell-fate decision in the developing fly eye (2). This observation was key in linking sina function to protein turnover. Sina homologs (Siah) were first isolated in mice (3), and found to be highly homologous across species. The human Siah family consists of Siah1 and Siah2, products of separate genes, with apparently distinct but overlapping functions. In mice, the Siah1 and Siah2 proteins are nearly identical to the human proteins, but there are two Siah1 forms, Siah1a and 1b, expressed from different genes (4).

Structure and Function

Structurally, the Siah family have a divergent *N*-terminal 40 to 80 residues, but are highly conserved through the remaining RING domain and C-terminal, substrate-binding domain (3). The crystal structure of the substrate-binding domain is homologous to tumor necrosis factor (TNF) receptor associated factor (TRAF) proteins (5) and subsequent studies showed that Siah2, via degradation of TRAF2, was a regulator of TRAF2 signaling (6). Siah family proteins can interact with a multitude of cellular proteins as diverse as scaffold proteins such as phyllopod, transcriptional repressors such as tramtrack and nuclear receptor co-repressor (NCoR), the motor protein Kid, the oncogene β-catenin, and the tumor suppressor TGF-β induced early gene (TIEG-1); a more extensive list can be found in ref. (7). In *Drosophila*, a ubiquitin ligase complex comprising Ebi, phyllopod, and sina interact to target tramtrack for ubiquitin-dependent proteolysis (8). In mammals, a similar ubiquitin ligase complex is formed, comprising Siah1, Ebi, and SIP (Siah interacting protein). Akin to the well-described anaphase promoting complex (APC) and Skp1/Cullin/F-box complex (SCF; ref. 9), the Siah/Epi/SIP complex functions in the ubiquitination-degradation of β-catenin (10). Siah contains a functionally important binding groove that recognizes a peptide motif within many substrates and adaptors (11). The crystal structure of a motif-containing peptide bound to Siah has been solved (12, 13), and competitive binding at this groove can inhibit Siah function *in vivo* (14).

Siah as a Tumor Suppressor Protein

Early reports suggested that Siah proteins, in particular Siah1, may function as tumor suppressor proteins on the basis of up-regulation in revertants of transformed cells (reviewed in ref. 15). Over-expression of Siah1 was also shown to cause growth arrest (16) or to be pro-apoptotic. Introduction of Siah1 into U937 cells suppressed the tumorigenicity of these cells when injected into *scid/scid* mice (15). Later experiments showed that Siah1 was involved in the degradation of the oncogene β-catenin, via interactions with the proteins adenomatous polyposis coli (APC) and Siah-interacting protein (SIP; refs. 10, 17). This noncanonical signaling pathway links β-catenin degradation to genotoxic stress and p53 activation (Fig. 1). If Siah proteins are tumor suppressors, mutation could be expected in human cancer but so far there is limited evidence for this, with only one report of a low frequency of inactivating mutations in gastric cancer (18) and a failure to find mutations in other cancer types (19).

Expression data has shown Siah2 to be strongly up-regulated in estrogen-receptor (ER)-positive breast tumors.[3] Previous data showed that Siah2 controls protein levels of the repressor of ER signaling, *N*-CoR (Fig. 1; ref. 20). Intuitively, inhibition of Siah2 should stabilize NCoR and thus inhibit ER signaling. A recent report, however, has shown that low levels of Siah2 expression correlate with more advanced, and tamoxifen-resistant ER-positive tumors (21).

Knockout Mice Define *In vivo* Roles

Given that Siah proteins have been found to bind and degrade a wide range of proteins, Siah1a and Siah2 gene-ablated mice have been valuable in establishing the significance of biochemical and cell biological studies. Siah1a null mice are subviable and display a meiotic block at metaphase I that impairs spermatogenesis and causes sterility in male mice (22). Although Siah2 null mice are outwardly normal in appearance (23), compound Siah1a/Siah2 mutation is lethal, implying a level of functional redundancy (23). There

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[3] D.D.L. Bowtell and A. Möller, unpublished observations.

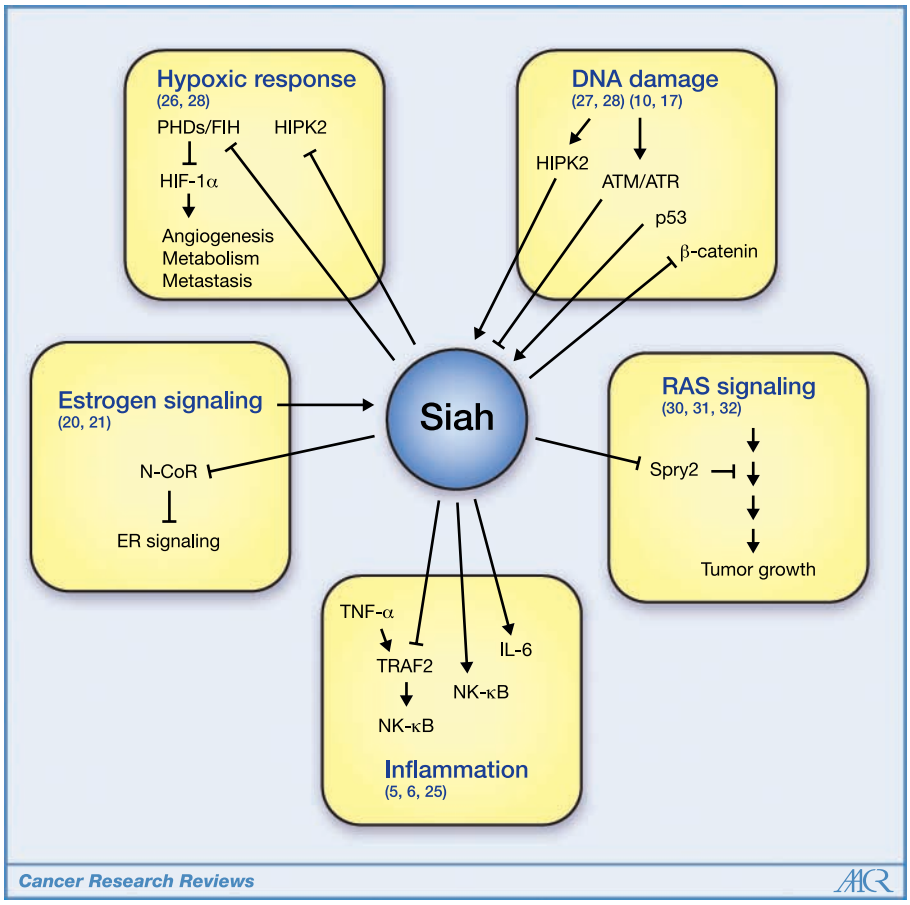

Figure 1. Siah proteins in cancer-related signaling pathways. Summary of aspects of the involvement of Siah proteins in cancer. Hypoxia: Siah proteins polyubiquitinate negative regulators of hypoxia signaling, PHD and FIH, to allow stabilization of HIF-1α under hypoxic conditions. Hypoxic conditions enhance the interaction of Siah and HIPK2, leading to HIPK2 degradation and derepression of hypoxia induced genes. DNA damage: Siah expression is induced by p53 and via a multiprotein complex regulates β-catenin degradation. The interaction of Siah-1 with the p53 kinase HIPK2 is destabilized by DNA damage, through the influence of ATM/ATR. Ras signaling: Loss of function experiments show that Siah proteins are effectors of Ras signaling and that inhibition of Siah reduces tumor growth in multiple models. Siah interacts with the negative regulator of Ras signaling, Spry2, resulting in its degradation. Inflammation: Siah interacts with Traf2, resulting in diminished NF-κB activation after TNF-α stimulation. Siah is required for innate immune responses in *C. elegans* and increases IL-6 and NF-κB levels. Estrogen signaling: Siah proteins are induced in human breast cancer cells by estrogen and degrade the co-repressor *N*-CoR, enhancing estrogen signaling. References are in brackets.

is no apparent increase in tumor incidence in Siah1a or Siah2 knockout mice, as might be expected with deletion of a tumor suppressor protein. Interestingly, contrary to previous studies linking Siah proteins with p53 signaling, Siah expression has been shown to be stable in mouse tissues and primary cells following activation of endogenous p53 by genotoxic stress (24). Importantly, p53 signaling, including up-regulation of p21, seemed normal in primary fibroblasts that lacked Siah genes (24). Recently, Siah1 has been reported as central to a conserved module involved in the innate immune response in *Caenorhabditis elegans* (25) and has been previously associated with immune signaling involving TRAF2 (6) and TNF-α (5).

Siah and the Hypoxic Response

One of the most striking roles for Siah proteins has been their demonstrated involvement in hypoxia signaling, via regulation of the key pro-angiogenic factor, HIF-1α. Siah proteins target for degradation prolyl hydroxylases (PHD) that are responsible for the post-transcriptional modification of HIF-1α, facilitating HIF-1α stabilization under hypoxic conditions (ref. 26 and reviewed in ref. 7). Siah2 knockout mice have a delayed and abrogated response to hypoxic conditions (26). At a cellular and tissue level, exposure of Siah2 mutants to hypoxia leads to significantly lower protein levels of HIF-1α, resulting in reduced hypoxia-induced gene expression (26).[4] These data suggest a potential for inhibition of the protumorigenic hypoxic response pathway by inhibition of Siah action (Fig. 1).

In two recent publications, a new layer of complexity to Siah's involvement in hypoxia and DNA damage has been added. The tumor suppressor HIPK2 has been shown to be a novel substrate of Siah proteins, whereby Siah1 (but not Siah2) is phosphorylated by ATM/ATR after DNA damage (27). This phosphorylation prevents the interaction of HIPK2 and Siah1, thereby stabilizing HIPK2, resulting in the induction of apoptosis. In a second study, Siah2 was shown to regulate HIPK2 levels under hypoxia (28), whereby HIPK2 directly phosphorylates Siah2 (but not Siah1) resulting in a disruption of the HIPK2/Siah2 interaction, thus stabilizing HIPK2 and promoting apoptosis (Fig. 1). Previously, HIPK2 has been shown to phosphorylate p53 at Ser46 (29), contributing to p53-mediated cell cycle arrest and apoptosis. The new findings that Siah proteins are potent negative regulators of HIPK2 open up the possibility that inhibition of Siah might result in elevated levels of HIPK2, thereby sensitizing cells to DNA damage-induced, p53-dependent apoptosis.

Siah and Ras Signaling in Cancer

Sina was first identified as being required downstream of Ras signaling in the *Drosophila* eye (1). Pancreatic tumors are characterized by very frequent k-Ras mutations and therefore provided an ideal model to test a requirement for Siah in Ras-driven cancer. Schmidt

[4] A. Möller, unpublished data.

and coworkers (30) showed that dominant-negative and shRNA inhibition of Siah potently impaired the growth of xenografts of human pancreatic tumor cells in mice. Erk phosphorylation (activation) was reduced, suggesting that Siah may be acting through the Ras/Mek/Erk pathway in these cells. Shown in a separate study, Siah2 causes the targeted degradation of Sprouty2 (31), a negative regulator of Ras signaling, providing a potential mechanism by which loss of Siah could impair Ras function. Siah expression is up-regulated in all major types of lung cancer compared with normal tissue (32). Again, use of a dominant negative Siah protein reduced lung cancer cell growth. Inhibition or knockdown of Siah2 reduced Erk signaling and cell proliferation, increased apoptosis, reduced colony formation in soft agar, and reduced tumorigenesis of A549 human lung cancer cells when injected into athymic nude mice (32).

Siah as a Therapeutic Target

Given the involvement of Siah proteins in hypoxia, estrogen, and Ras signaling, blocking Siah function could be an attractive approach to impairing angiogenesis and proliferation. Interfering with Hif-1 up-regulation through Siah inhibition is appealing, as it may more broadly impact on the tumor's adaptive responses to hypoxia than inhibition of individual downstream Hif-1 effectors, such as VEGF.

Proof-of-concept for small molecule inhibition of Siah has been obtained with a short protein fragment that binds competitively with high affinity to the substrate binding site of Siah proteins, and resulted in reduced growth of breast cancers (14) and reduced frequency of metastases in melanoma (33). In the breast cancer model (14), Siah seemed to be functioning, at least in part, by inhibition of the hypoxic-response pathway, as work with the isolated cells showed reduced induction of HIF-1α target genes and tumors displayed reduced angiogenesis. Importantly, whereas blockade of substrate binding inhibited HIF-1 responses, use of a dominant negative Siah protein also influenced Ras signaling (33), suggesting that different parts of the protein may need to be inhibited to fully modulate Siah function.

The above studies highlight three methods of Siah inhibition, with some different biological effects. The main options for Siah inhibition seem to be disruption of protein-protein interactions (between Siah and substrate or E2), disruption of RING domain integrity (inhibiting Siah/E2 interaction), or inhibition of gene expression. Siah proteins have been shown to have two important functional domains, an E2-binding RING domain and a C-terminal substrate-binding domain (34), though some substrate interactions map to or near the RING domain (31, 35). The relative specificity of inhibitor compounds targeted to each of these regions remains to be determined. Siah1 and Siah2 are highly homologous in the SBD but vary in the *N*-terminus/RING domain. Inhibitors directed to the SBD may be specific for Siahs, but inhibit both Siah1 and Siah2, whereas inhibitors targeted to the *N*-terminus/RING may offer Siah1/Siah2 selectivity, but have the potential to also inhibit other RING-containing ubiquitin ligases.

Summary

Recent work indicates that Siah proteins are promising novel therapeutic targets in cancer. Siah proteins are involved in several signaling pathways, including Ras, hypoxia, estrogen, DNA damage, and others, and inhibition of Siah proteins seems to slow cancer growth and/or reduce metastatic spread. The data in tumor models so far focus on inhibition or loss of Siah proteins in cancer cells only. It is still not clear what the effects of inhibition of Siah proteins in the whole adult animal will be. The double knockout mouse (Siah1a and Siah2) dies at birth for unknown reasons, though the role of Siah in response to changes in oxygen tension may be important. There is need for a better understanding of the differences between Siah1 and Siah2 functions. For many of the Siah substrates studied, both Siah1 and Siah2 can function similarly, though usually work focusing on only one form is reported. The role of Siah proteins as tumor suppressors is at odds with more recent data, although it should not be ignored. The impact of targeting various parts of the Siah proteins on different signaling pathways also deserves attention in the drive to develop small molecule inhibitors of this protein family.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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