

Siah: A Promising Anticancer Target

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Abstract

Siah ubiquitin ligases play important roles in a number of signaling pathways involved in the progression and spread of cancer in cell-based models, but their role in tumor progression remains controversial. Siah proteins have been described to be both oncogenic and tumor suppressive in a variety of patient cohort studies and animal cancer models. This review collates the current knowledge of Siah in cancer progression and identifies potential methods of translation of these findings into the clinic. Furthermore, key experiments needed to close the gaps in our understanding of the role Siah proteins play in tumor progression are suggested. *Cancer Res*; 73(8); 2400–6. ©2013 AACR.

Introduction

The proteasome controls protein abundance within the cell through proteolytic degradation of unneeded or damaged proteins. Most proteins are targeted for degradation by polyubiquitination mediated by E3 ubiquitin ligases (1, 2). The proteasome inhibitor, bortezomib, is used as a single agent or in combination with conventional therapies in the treatment of multiple myeloma and shows clinical efficacy as a novel anticancer drug. However, bortezomib blocks all protein proteolysis by the proteasome without discrimination, causing various systemic toxicities and the development of resistance (1). Treatment with bortezomib shows little to no efficacy against solid tumors and is limited to hematologic malignancies (1). E3 ubiquitin ligases are therefore a more specific and effective target for drug development in comparison with general proteasome inhibitors to reduce treatment side effects and increase efficacies (1).

Siah proteins, evolutionarily conserved E3 RING zinc finger ubiquitin ligases, have recently been implicated in various cancers and show promise as novel anticancer drug targets. Humans have 2 Siah proteins, Siah1 and Siah2, derived from the *SIAH1* and *SIAH2* genes, respectively. Mice, on the other hand, have 3 Siah proteins, Siah1a, Siah1b, and Siah2 (3). Siah mediates its E3 ubiquitin ligase activity by directly binding to its substrates (4) or by acting as the essential RING domain subunit of a larger E3 complex (5) and can form homodimers and heterodimers. Siah binds to a highly conserved PxAxVxP motif found in most substrates via its conserved substrate-binding domain (SBD; refs. 6, 7). The various substrates of Siah have recently been reviewed and are discussed elsewhere (8, 9).

Little is known about the upstream regulatory mechanisms of Siah proteins. Mitogen-activated protein kinase (MAPK) p38

phosphorylates Siah2 under hypoxic conditions, causing it to relocate to the cytoplasm and subsequently increases its ubiquitin-targeted degradation activity (10). In addition, the deubiquitinating enzyme USP13 reduces the substrate degradation activity of Siah proteins (11). Furthermore, in estrogen receptor (ER)-positive breast cancer cells, estrogen increases Siah2 levels, resulting in degradation of the nuclear receptor corepressor 1 (N-CoR) and a reduction in N-CoR-mediated repressive effects on gene expression, thus promoting cancer growth (12).

Cancer cell lines have been used to identify several roles for Siah in cancer progression pathways, including the Ras, p53, and hypoxic response signaling pathways and are reviewed in depth elsewhere (reviewed in ref. 8).

In this review, we discuss the roles of Siah proteins in cancer progression. Reports identifying a role for Siah in cancer progression in animal models and patient cohorts are collated in Table 1. We also discuss the evidence for oncogenic and tumor-suppressive functions of Siah in patients with cancer with the aim of suggesting future key experiments to elucidate the potential clinical applications of targeting Siah in cancer therapy.

Siah inhibition in mouse tumor models

In recent years, our understanding of the role of Siah in cancer has vastly increased by verifying cell-based findings in animal models (Table 1). The function of Siah in tumorigenesis was first investigated in pancreatic cancer (Table 1; ref. 13). Expression of a dominant-negative, RING-mutated version of Siah1 and Siah2 in human pancreatic cancer cells led to reduced tumor growth in nude mice (Table 1; ref. 13). This work was the first to show that targeting Siah could attenuate oncogenic Ras signaling to reduce tumor growth (Table 1; ref. 13). This work was validated in a subsequent study in lung cancer (14), suggesting a similar tumor-promoting role for both Siah1 and Siah2.

Around the same time, inhibition of Siah was found to have antitumorigenic functions in melanoma and breast cancer. In addition to using dominant-negative RING mutants for Siah1 and Siah2, Siah substrate binding was blocked using a peptide inhibitor, Phyllopod (PHYL; ref. 15). Inhibition of Siah substrate

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binding through PHYL reduced metastatic spread by attenuating signaling via the hypoxic response pathway. In contrast, blocking E3 activity using RING mutants primarily reduced tumor growth by affecting Sprouty2 in the Ras signaling pathway (Table 1; ref. 15). These data confirmed a role for Siah in the Ras signaling pathway and identified a new role for its regulation of the hypoxic response pathway in solid cancers. Similar findings were obtained in a syngeneic, orthotopic breast cancer model, in which the PHYL-mediated inhibition of Siah substrate binding reduced tumor growth and angiogenesis (Table 1; ref. 16). The findings in melanoma and breast cancer suggest that simultaneous inhibition of all Siah proteins in tumor epithelial cells alone may be sufficient to attenuate tumor growth.

Two recent studies investigated the impact of genetic knockout of Siah2 in spontaneous models of prostate and breast cancer. In the Tramp model of neuroendocrine prostate cancer, the genetic knockout of Siah2 restricted prostate tumor progression to the atypical hyperplasia stage, preventing progression to advanced tumor stage and reducing metastasis (Table 1; ref. 17). Loss of Siah2 attenuated Hif-1 α protein levels, decreased proliferation, and increased apoptosis in these tumors. Similarly, in the Polyoma Middle T (PyMT)-driven model of breast cancer, loss of Siah2 delayed tumor onset, reduced stromal infiltration into the tumor microenvironment, and resulted in a normalized tumor vascular morphology (Table 1; ref. 18). Exploiting this vascular normalization phenotype, increased delivery of standard chemotherapeutic drugs was achieved in Siah2 knockout tumors, prolonging the survival of these mice (18).

The work summarized above depicts our current understanding of Siah in cancer model systems. Although it is a little premature at this stage to draw conclusions from such a wide array of models and approaches, it is interesting to note that all 6 reports support an oncogenic role of Siah proteins in cancer progression through 2 main pathways, the Ras signaling pathway (lung, pancreatic, and melanoma cancer models; Table 1) and the hypoxic response pathway (prostate, melanoma, and breast cancer models; Table 1). Therefore, Siah may act through both pathways simultaneously or through different pathways at different stages of tumor progression, as exemplified in the melanoma model (Table 1; ref. 17).

Siah1 and Siah2 in cancer patients

Currently, only a limited number of studies link expression of Siah genes and proteins with disease progression in human cancer (comprehensively listed in Table 1). These studies present a contradicting mix of results and opinions on the classification of Siah as an oncogene or a tumor suppressor, as discussed below.

Siah as an Oncogene

Two recent studies of breast cancer sections provide evidence for an oncogenic role for Siah proteins (Table 1; refs. 19, 20). An antibody capable of detecting both Siah1 and Siah2 showed Siah protein levels were significantly increased in ductal carcinoma *in situ* (DCIS) tumor tissue compared with normal adjacent tissues (20). In addition, tumor samples from

patients with disease recurrence had higher Siah expression than those from patients without recurrence, suggesting that Siah could be used as a prognostic biomarker to predict DCIS progression to invasive breast cancer (Table 1; ref. 20). Importantly, however, this study did not discriminate between Siah1 and Siah2 expression, and it is therefore not conclusive whether both or only one Siah protein is a valuable prognostic marker. In another study, nuclear Siah2 protein levels were found to progressively increase from normal breast tissue samples through to DCIS and invasive breast cancer samples (Table 1; ref. 19), suggesting that Siah2 might be more useful as a predictive marker. Of particular interest was the significant increase and positive association of Siah2 nuclear staining with invasive breast carcinoma samples, particularly with basal-like breast cancers (Table 1; ref. 19). These findings are reflected by increased correlation of Siah2 with high-grade, malignant human prostate cancers but not low-grade tumors (17). Furthermore, in patients with hepatocellular carcinoma (HCC), nuclear accumulation of Siah1 and Siah2 were correlated with hepatocarcinogenesis and tumor dedifferentiation (21, 22). Together, these reports describe oncogenic functions for Siah proteins, especially Siah2, in breast, prostate, and liver cancer and highlight an association between increased Siah2 levels and more malignant and invasive stages of cancer. It is very interesting to note that nuclear Siah seems to be specifically connected in these reports with the oncogenic roles, and the impact of subcellular localization of Siah proteins on its function warrants thorough investigation in the future.

Tumor-Suppressor Role

Early studies suggested tumor-suppressive roles for Siah, especially Siah1. *In situ* hybridization on cancer tissue microarrays revealed that gene expression of both SIAH1 and SIAH2 were downregulated in human breast cancer, correlating with decreased overall disease-free survival of patients (Table 1; ref. 23). This is in stark contrast with elevated Siah2 protein levels being associated with reduced survival and disease progression as discussed above (19, 20), showing an apparent disconnect between the ability to predict outcomes solely based on Siah gene or protein abundance.

Siah1 has also been shown to be decreased in human breast cancer tissue compared with normal tissue, suggesting it either functions as a tumor suppressor or its loss is associated with tumor progression (24). In other cancers, such as gastric and liver cancer, low levels of Siah1 have been shown to reduce apoptosis, thereby promoting cancer progression (25, 26). Together these studies and those listed in Table 1 allude to a tumor-suppressive role for Siah1 proteins in various cancer types.

Exploring the different roles of Siah1 and Siah2 in cancer

It might be expected that the highly conserved genetic and protein sequences of Siah1 and Siah2 would reflect similar roles and functional redundancy in cancer progression. However, as discussed, Siah1 is more often described as a tumor suppressor and Siah2 an oncogene. So how can we explain this discrepancy?

Table 1. The oncogenic and tumor-suppressor roles of Siah proteins in *in vitro* and *in vivo* models of cancer

Siah isoform	Role	Cancer type	Effect	Reference
Animal models of cancer				
Siah (isoform not specified)	Oncogene	Xenograft mouse model of pancreatic cancer	Siah mediates Ras signaling in pancreatic tumor progression	(13)
All Siah	Oncogene	Mouse model of breast cancer	Inhibition of Siah with PHYL reduced tumor growth	(16)
Siah2	Oncogene	Mouse model of melanoma	Inhibition of Siah2 activity reduces melanoma progression via HIF-dependent and -independent pathways	(15)
Siah2	Oncogene	Xenograft mouse model of lung cancer	Disrupting Siah2 function in lung cancer induced apoptosis and prevented tumor growth	(14)
Siah2	Oncogene	Mouse model of melanoma	Siah2 inhibition with vitamin K3 blocks melanoma progression by disrupting hypoxia and MAPK signaling	(37)
Siah2	Oncogene	Mouse model of spontaneous prostate cancer	Reduced tumor progression and metastasis in Siah2-null prostate cancer mice (Siah2-dependent regulation of Hif and FoxA2 interaction)	(17)
Siah2	Oncogene	Mouse model of spontaneous breast cancer	Siah2-knockout mice show delayed breast tumor onset, reduced stromal infiltration, and altered tumor angiogenesis	(18)
Human tumor tissue sample and cancer cell line studies				
Siah (isoform not specified)	Oncogene	Human breast cancer DCIS	High Siah expression predicts DCIS progression to invasive breast cancer	(20)
Siah2	Oncogene	Human prostate cancer	Increased Siah2 expression in human NE prostate tumor samples	(17)
Siah2	Oncogene	Human breast cancer	Siah2 upregulation in basal-like breast cancers	(19)
Siah2	Oncogene	Human HCC	Nuclear accumulation of Siah2 enhances motility and proliferation of liver cancer cells	(22)
Siah1	Oncogene	Human HCC	Nuclear accumulation of Siah1 induces proliferation and migration of liver cancer cells	(21)
Siah1	Oncogene (?)	Human HCC	Siah1 expression levels in HCCs is low, but further reduction by siRNA decreased tumor cell viability	(38)
Siah1 and Siah2	Tumor suppressor	Human breast cancer	Low levels of Siah1 and Siah2 correlated with decreased probability of disease-free survival	(23)
Siah1 and Siah2	Tumor suppressor	Human leukemia	Siah mediates elimination of oncogenic proteins through proteasomal and kinase signaling pathways in leukemia	(27)

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Table 1. The oncogenic and tumor-suppressor roles of Siah proteins in *in vitro* and *in vivo* models of cancer (Cont'd)

Siah isoform	Role	Cancer type	Effect	Reference
Siah1/Siah1L splice variant	Tumor suppressor	Human breast cancer cells	Overexpression enhances radiosensitivity	(39)
Siah1	Tumor suppressor	Human breast cancer tissues and cells	Decreased Siah1 expression in human breast cancer tissue and cells, mirrored by decreased Kid/KIF22	(24)
Siah1	Tumor suppressor	Human breast cancer tissues and cells	Downregulation of Siah1 observed in human breast cancer tissue, <i>SIAH1</i> -induced apoptosis dependent on Bim	(40)
Siah1	Tumor suppressor	Human breast cancer cell lines	Overexpression of <i>Siah1</i> induced apoptosis via JNK(/Bim) pathway; suppression of <i>SIAH1</i> increased invasion via ERK pathway	(41)
Siah1	Tumor suppressor	Human leukemia and breast cancer cell lines	Siah1-transfected cancer cells have a suppressed malignant phenotype	(29)
Siah1	Tumor suppressor	Gastric cancer	Inactivating mutations in the <i>SIAH1</i> gene prevented apoptosis and may allow gastric cancer progression	(26)
Siah1	Tumor suppressor	Human liver cancer cells	Siah1 induces apoptosis by reducing β -catenin and PEG10	(25)

Abbreviations: DICS, ductal carcinoma *in situ*; ERK, extracellular signal-regulated kinase; HCC, hepatocellular carcinoma; NE, neuroendocrine.

Both ubiquitin ligases have a number of substrates in common (reviewed in refs. 11, 12), but there are some proteins targeted by one and not the other, which might affect different cellular signaling networks. A recent comprehensive review describes the role of Siah-mediated targeted protein degradation of leukemia-relevant substrates (27), highlighting the necessity for more in-depth understanding of the different Siah protein functions before designing Siah-based therapeutic strategies.

The question remains as to whether there truly is a difference in the oncogenic and tumor-suppressive roles of Siah in cancer from one patient to another or one cancer type to another. Recently, one study in HCC reported that nuclear protein accumulation of Siah1 and Siah2 were weakly and inversely correlated, suggesting potentially exclusive expression patterns (22). This has so far been the only study to address what happens to the second Siah protein in the same cancer or cohort. To truly understand the role Siah plays in cancer, and if it is a valid anticancer target, studies should interrogate whether Siah1 and Siah2 are coregulated, inversely expressed, or completely independent from each other in various cancer types. Because of an apparent disconnect between gene and protein expression (Table 1), results from a well-annotated cancer tissue array should ideally be paired up with corre-

sponding gene expression analysis. Furthermore, depending on the cancer, cancer subtype, and site of metastasis, Siah1 and/or Siah2 may have opposing roles. Assessment of the gene and protein expression of Siah1 and Siah2 in each sample will provide valuable information as to whether one or both Siah proteins are suitable anticancer drug candidates in a certain cancer stage and subtype. Siah1 has been described to cause tumor reversion (28, 29; reviewed in ref. 30). If this is a Siah1-specific effect, inversely correlated or independent results for Siah2 need to be investigated as well. Importantly, posttranslational modifications of Siah proteins have also been shown to change the activity, substrate specificity, and subcellular localization of Siah (10, 27, 31). Therefore not only the abundance, but also the extent of modifications and function of Siah proteins as a result need to be taken into consideration.

Furthermore, altered genomic stability is associated with most cancers. Siah1 is located on chromosome 16q12.1, which has been reported to be deleted in 30% of all HCCs and in a variety of other cancers (32). In contrast, genomic copy number gains have been reported for Siah2, located on chromosome 3q25.1, in basal-like breast cancer (19). Thus, more studies investigating genomic changes in Siah1 and Siah2, their gene expression, protein abundance and localization, and functional impact on cancer progression are needed.

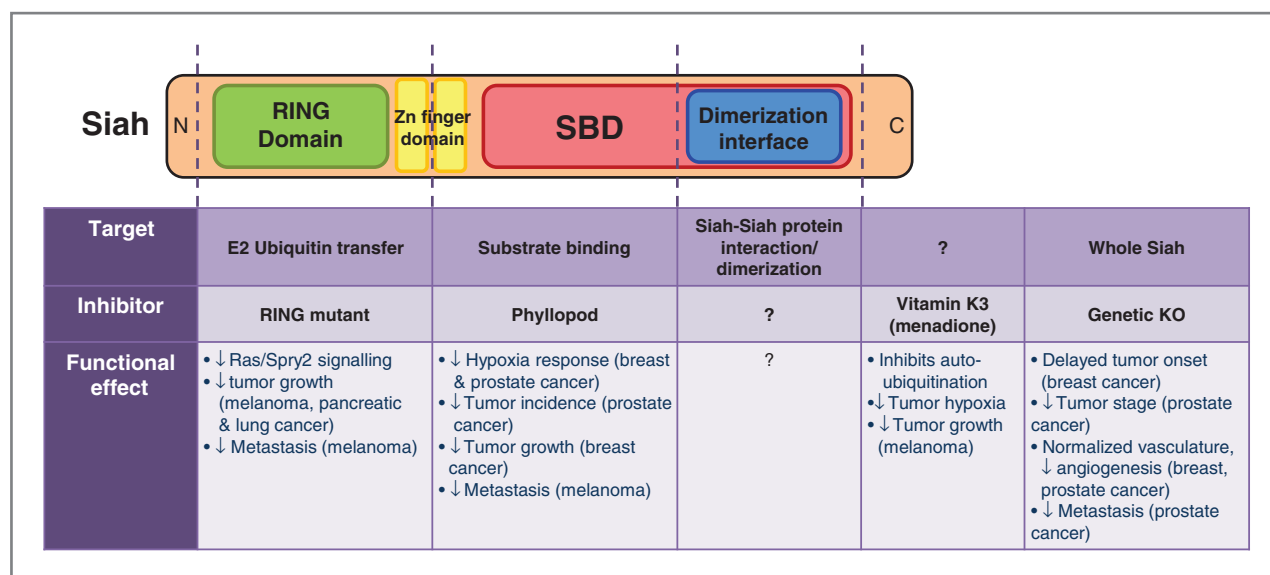


Figure 1. Summary of the targeting options for the Siah protein and the described functional effects on cancer progression. KO, knockout.

Clinical Relevance—Therapeutic Strategies

Although initial, cell-based observations describe Siah as either an oncogene or tumor suppressor, the use of whole-animal-based model systems uniformly supports an oncogenic role of Siah proteins in different cancers (Table 1). In patient cohort studies, the majority of reports support the notion of Siah2 as an oncogene and Siah1 as a tumor suppressor (Table 1). Thus, specific inhibitors for each Siah isoform may be necessary.

How could we achieve a blockade of Siah?

Siah has 3 likely sites for intervention—these being interference with the E3 ubiquitin ligase function (Fig. 1; ref. 33), the SBD, and the Siah–Siah dimerization domain (Fig. 1; refs. 6, 34). An inhibitor of Siah should disrupt the hypoxia signaling pathway to prevent malignant transformation and spread of cancer, which can be achieved by blocking the SBD as shown using PHYL. As described above, PHYL is capable of reducing tumor growth and metastasis in various models (15–17). Because of high homology in the SBD, however, the development of a drug targeted specifically to Siah1 or Siah2 will be difficult.

The N-terminal RING domain of Siah proteins promotes the proteolysis of specific target proteins via the ubiquitin–proteasome pathway (2, 33). Interference with the RING domain impairs Ras signaling and thereby interferes with cancer progression. The N-terminal regions of Siah1 and Siah2 differ from each other (35), making it possible to design Siah1- and Siah2-specific RING domain inhibitors. However, interference with the RING domain does not impair the ability of Siah to bind to substrate proteins via the SBD or to form Siah1/Siah2 heterodimers (15, 33).

A third way to inhibit Siah would be to disrupt the dimerization site between 2 Siah monomer proteins (Fig. 1). The

protein Zyxin has been described recently to inhibit Siah1/Siah1 homodimerization (36), although the impact on Siah1/Siah2 heterodimers or Siah2/Siah2 homodimers was not assessed. In addition, the functional consequences of preventing Siah homo- or heterodimerization in cancer are yet to be explored.

The only drug targeting Siah described so far, vitamin K3 (menadione), was identified as an inhibitor of Siah2 ubiquitin ligase activity in a screen of U.S. Food and Drug Administration–approved therapeutic drugs (37). Menadione inhibits both arms of the Siah2 downstream signaling network, the Ras/MAPK pathway and the hypoxic response pathway (37), suggesting that it might act through the SBD and RING domain (Fig. 1). Mice treated with menadione had reduced tumor growth rates associated with decreased Hif-1 α stabilization (37). Although the mechanism of Siah2 inhibition of menadione has not been entirely elucidated, this study provides evidence for the screening and functional validation of Siah inhibitors to prevent cancer progression.

Conclusions

Collectively, *in vitro*, *in vivo*, and patient sample studies describe contradictory roles for Siah1 and Siah2 in cancer progression, metastasis, and therapeutic responses. Evidence from animal models and patient cohort studies strongly suggests that Siah2 acts in an oncogenic fashion, whereas Siah1 often functions as a tumor suppressor. In various *in vivo* models of cancer, targeting the SBD or RING domain of the Siah gene reduces tumorigenesis and/or metastasis. Inhibitors targeting specific regions of either or both Siah proteins may be necessary to fine-tune this therapeutic approach. Because Siah plays central roles in key cancer-related signaling pathways, the modulation of the activity or abundance of this key molecule will allow novel

preclinical and clinical treatment modalities for patients with cancer in the future.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: C.S.F. Wong, A. Möller

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