When the Sphingosine Kinase 1/Sphingosine 1-Phosphate Pathway
Meets Hypoxia Signaling: New Targets for Cancer Therapy

Isabelle Ader,1,2 Bernard Malavaud,1,2,3 and Olivier Cuvillier1,2

1CNRS, Institut de Pharmacologie et de Biologie Structurale; 2Université de Toulouse, UPS, IPBS; and 3CHU Toulouse, Hôpital Rangueil, Toulouse, France

Abstract

The reduction in the normal level of tissue oxygen tension or hypoxia is a characteristic of solid tumors that triggers the activation of signaling pathways promoting neovascularization, metastasis, increased tumor growth, and resistance to treatments. The activation of the transcription factor hypoxia-inducible factor 1α (HIF-1α) has been identified as the master mechanism of adaptation to hypoxia. In a recent study, we identified the sphingosine kinase 1/sphingosine 1-phosphate (SphK1/S1P) pathway, which elicits various cellular processes including cell proliferation, cell survival, or angiogenesis, as a new modulator of HIF-1α activity under hypoxic conditions. Here, we consider how the SphK1/S1P signaling pathway could represent a very important target for therapeutic intervention in cancer. [Cancer Res 2009;69(9):3723–6]

Background

Hypoxia, the lack of oxygen in which oxygen delivery does not meet demand, is a characteristic hallmark of locally advanced solid tumors. Indeed, it has been estimated that up to 50% to 60% of solid tumors contain areas of hypoxic tissues as a consequence of an imbalance between oxygen supply and consumption in proliferating tumors because the developing new blood vessels are aberrant and have poor blood flow (1). Even though hypoxia is toxic to both cancer and normal cells, cancer cells undergo adaptive changes that allow them to survive and proliferate by activating pathways that stimulate angiogenesis to increase oxygen supply and by activating metabolic pathways that permit adaptation to the reduced oxygen accessibility (2). These mechanisms contributing to a malignant phenotype characterized by relentless tumor expansion, increased risk of metastasis, angiogenesis, and development of resistance to therapies, are coordinated transcriptionally by the hypoxia-inducible factors HIF-1α, HIF-2α, and HIF-3α transcription factors. HIF-1α has been identified as the master regulator of the response of mammalian cells to hypoxia. HIF-1α is a heterodimeric transcription factor, composed of a constitutively expressed nuclear β subunit (also called ARNT1) and an oxygen sensitive α subunit (3). Under well-oxygenated conditions, the posttranslational hydroxylation of one or two prolyl residues of HIF-1α is mediated by members of the oxygen-dependent specific prolyl hydroxylase domain (PHD) family (4). The hydroxylation of HIF-1α is required for its recognition by the von Hippel-Lindau tumor suppressor gene product (pVHL) of the E3 ubiquitin ligase complex, followed by its ubiquitination and proteasomal degradation. Under low oxygen conditions or in cells lacking functional pVHL (renal cell carcinomas and other tumors regrouped in the VHL syndrome), HIF-1α remains unhydroxylated, and therefore accumulates, then translocates to the nucleus where it heterodimerizes with its partner HIF-1β. The binding of the heterodimeric HIF-1 to hypoxia response elements (HREs) located in the promoter region of its numerous target genes like those encoding angiogenesis-promoting factors such as vascular endothelial growth factor (VEGF) or platelet-derived growth factor, glucose transporters, enzymes of glycolytic pathway, in addition to proteins involved in extracellular matrix remodeling, cell proliferation, or survival (5) leads toward a surviving phenotype with clinical aggressiveness.

Overwhelming evidence based on immunohistochemical studies of human tumor sections indicates that HIF-1α is overexpressed in the majority of human cancers. In most cases, there is a direct correlation between the degree of expression of HIF-1α or some of its prominent and specific downstream targets such as the glucose transporter GLUT-1 or the carbonic anhydrase IX and poor clinical outcomes: poor response to radiation and chemotherapy, more aggressive and invasive tumors, and increased patient mortality (1). In preclinical models, HIF-1α overexpression is an accelerating factor in tumor progression and metastasis (6), whereas inhibition of HIF-1α activity impedes tumor growth and angiogenesis (7), supporting proof-of-principle that HIF-1α inhibitors could have therapeutic benefit.

When the Sphingosine Kinase 1/Sphingosine 1-Phosphate Pathway Meets Hypoxia Signaling

Sphingosine 1-phosphate (SIP) is a potent lipid mediator regulating a broad variety of cellular processes such as cell proliferation, apoptosis, calcium homeostasis, vascular maturation, or angiogenesis (8). SIP content in cells is low and strictly kept under control by a tightly regulated balance between its synthesis and its degradation. The balance between the intracellular levels of SIP and its metabolic precursors ceramide and sphingosine is regarded as a switch that could determine whether a cell proliferates or dies (9). A decisive regulator of this sphingo lipid rheostat is the sphingosine kinase 1 (SphK1), which generates SIP from its metabolic precursor sphingosine. SphK1 activity can be rapidly stimulated by many agonists (e.g., growth factors such as PDGF, FGF, EGF, HGF, VEGF, etc.), thus reflecting its critical role in controlling SIP levels. Once generated, SIP can act either extracellularly by binding to one of the five cell surface G-coupled SIP receptors to drive paracrine or autocrine signaling cascades or intracellularly by a mechanism still unknown (8). Importantly, the agonist-induced SIP production as well as its downstream effects can be impeded by inhibition of the SphK1 gene expression or enzymatic activity demonstrating that SphK1 plays a crucial role in the observed effects ascribed to SIP. Multiple studies support the
convincing role of SphK1 in the promotion of oncogenesis in addition to being a cellular target for many anticancer treatments. On the one hand, since the demonstration of its oncogenic nature, SphK1 expression has been found up-regulated in a number of solid tumors, and high SphK1 expression in glioblastoma and breast cancer has been correlated with poor survival of patients (10). On the other hand, anticancer regimens (chemotherapies, radiation therapy) have been shown to down-regulate SphK1 activity in various cancer cell and animal models, suggesting that SphK1 could act as a “sensor” to anticancer therapies, whereas its enforced expression can protect cancer cells from apoptosis (10).

Accumulating evidence has implicated the S1P metabolism in hypoxia in normal cells. Indeed, we and others have shown the involvement of SphK1 in the adaptation of cardiomyocytes to ischemia both in vitro and in animal models (11, 12), and SphK1-null cardiomyocytes have been shown to be more susceptible to hypoxia (13). Moreover, increased proliferation of smooth muscle cells induced by hypoxia relies on the generation of S1P (14), and increased SphK1 expression was reported in pulmonary smooth muscle cells under both acute and chronic hypoxia (15). Unexpectedly, given the significance of hypoxia in cancer, the association between the SphK1/S1P signaling and adaptation to hypoxic conditions was only investigated very recently in tumor cells. Indeed, we have shown for the first time that SphK1 could regulate HIF-1α accumulation under low oxygen tension (16). Done in five distinct tumor cell models (glioblastoma, prostate, breast, lung, kidney), our studies suggest a canonical role for SphK1 in cancer adaptation to hypoxia. A sharp stimulation of SphK1 activity occurred rapidly (within 1-2 hours) but transiently under hypoxic conditions indicating a likely posttransductional effect, with SphK1 activation invariably preceding HIF-1α accumulation. Interestingly, SphK1 stimulation seemed to depend on reactive oxygen species (ROS) production because the ROS scavenger N-acetyl cysteine was able to prevent both SphK1 stimulation and HIF-1α accumulation. How generation of ROS results in SphK1 stimulation is currently unknown. A large body of evidence suggests that ROS can modulate HIF-1α level (4) through direct inhibition of prolyl-hydroxylases, but also indirectly via activation of the Akt/GSK3β signaling (17). Interestingly, we found that the SphK1-mediated accumulation of HIF-1α levels under hypoxia relied on the Akt/GSK3β pathway (Fig. 1). How the biolipid S1P produced upon SphK1 stimulation activates Akt/GSK3β signaling (intracellular versus autocrine effect) is currently under investigation. Finally, demonstrating the instrumental role of SphK1, both

Figure 1. Regulation of HIF-1α level by the SphK1/S1P signaling pathway in cancer cells subjected to hypoxia. Under low oxygen tension (1% CO₂), SphK1 activity is quickly but transiently stimulated by an unknown mechanism relying on reactive oxygen species (ROS) production. S1P, either intracellularly or through the binding to one of its G-coupled receptor (S1PRs), triggers activation of the Akt/GSK3β signaling that regulates HIF-1α level. Inhibition of the SphK1 gene expression or enzymatic activity causes the down-regulation of the Akt/GSK3β signaling leading to the degradation of HIF-1α by the von Hippel-Lindau tumor suppressor protein (pVHL)-mediated ubiquitin proteasome machinery.
pharmacological and RNA-silencing inhibition of SphK1 activity could prevent activation of Akt/GSK3β signaling, accumulation of HIF-1α and its transcriptional activity in all human cancer cell lineages. Since publication of the seminal report by Wang and colleagues, the major regulatory mechanism of HIF-1α accumulation under hypoxia is its pVHL-mediated proteasomal degradation (18). We established that HIF-1α degradation triggered by SphK1 inhibition was controlled by the proteasome via a pVHL-dependent mechanism as shown by inhibition of the proteasome by the MG132 compound or using pVHL-deficient and reconstituted pVHL cell models (ref. 16) (Fig. 1).

Whereas initial characterization of hypoxia-induced transcription focused on HIF-1α, there is now evidence that HIF-2α, which is closely related to HIF-1α, could regulate unique genes and physiological functions. HIFα subunits differ in expression profiles, with HIF-1α ubiquitously expressed and HIF-2α limited to endothelium, kidney, heart, lung, gastrointestinal epithelium, and some cells of the central nervous system (19). So far, distinct roles for HIF-1α versus HIF-2α in promoting tumor growth have been most clearly defined in von Hippel-Lindau disease-associated clear cell renal carcinoma (ccRCC) (ref. 20), which can produce either HIF-1α and HIF-2α or HIF-2α alone, and in which the role for HIF-2α as a driver of a more aggressive disease has been recently highlighted (21). Interestingly, a recent report has suggested a link between the SphK1/SIP pathway and HIF-2α in cobalt chloride (CoCl2) chemically induced hypoxia in glioma-derived U87 cells (22). Under these conditions, increases in SphK1 message, protein expression, and enzyme activity were noted. Knockdown of HIF-2α by RNA interference abolished the induction of SphK1, whereas HIF-1α down-regulation resulted in increased HIF-2α and SphK1 expression. In terms of regulatory mechanisms, direct binding of HIF-2α to the SphK1 promoter was suggested (22). Although in apparent contradiction with our own data in which SphK1 activity was found to be an upstream regulator of HIF-1α in the same cell model, it should be noted that the influence of SphK1 activity on HIF-1α protein levels was not investigated in that study. In addition, hypoxia mimetics such as CoCl2 should not be considered equivalent to bona fide hypoxia, and results should be interpreted accordingly. Nevertheless, it cannot be ruled out that SphK1 activity might first regulate HIF-1α (and/or HIF-2α) activity, which in turn could transcriptionally regulate the proangiogenic and prosurvival SphK1/SIP pathway. Additional studies are required to elucidate whether (1) SphK1 can be a target gene of HIF-2α as proposed by Obeid and co-workers (22), and (2) SphK1 activity regulates HIF-2α as it does for HIF-1α (16), particularly in a relevant model such as the von Hippel-Lindau-associated clear cell renal carcinoma (ccRCC).

New Targets for Cancer Therapy

Drugs aimed at targeting tumor stromal-cell responses represent a novel category of therapeutic agents. As a matter of fact, a large number of drugs are currently in clinical trials as anticancer agents on the basis of their ability to inhibit angiogenesis. For instance, therapies against VEGF, an HIF-1 target, have shown some efficacy and have prompted interest in targeting global HIF-1 activity. Given the central role of HIF-1, it is clear that decreasing its activity could represent a valid strategy to control tumor hypoxia and its molecular consequences: increased potential for invasion, neoangiogenesis, metastasis, and patient mortality. Finding a specific HIF-1 inhibitor is not easy as transcription factors are conventionally considered difficult if not impractical targets for the discovery of small molecule inhibitors (23). Signal transduction pathways involved in HIF-1 stabilization occurring during hypoxic stress can also be targeted to inhibit HIF-1 activity. Although they lack selectivity, several agents that inhibit signal transduction pathways regulating HIF-1 activity, angiogenesis, and xenograft growth have been identified (e.g., PI3K/AKT inhibitors, camptothecins, etc.) (refs. 7, 24). As SphK1 can act as a master regulator of HIF-1 activity, we suggest its inhibition as a novel and valid approach to control tumor hypoxia and its molecular consequences. In human tumors, increased activity of HIF-1 is induced by physiological stimulation as well as genetic alterations such as PTEN or p53 loss-of-function mutations. Interestingly, we have established that SphK1 inhibitory strategies were able to almost abrogate HIF-1α expression in cancer cells regardless of their PTEN or p53 status (e.g., prostate PC-3 cells are null for p53 and PTEN; glioblastoma U87 cells contain wild-type p53 but are null for PTEN; lung A549 cells contain wild-type p53 and PTEN).

Activation of HIF-1α expression in tumors seems to be initiated through a vicious cycle of induction of poorly functioning vasculature perpetuating the development of a hypoxic microenvironment throughout the tumor. Hypoxic cancer cells are prone to be more resistant to radiation and chemotherapy. In addition to drugs developed specifically as antiangiogenesis agents such as the anti-VEGF strategies (25), it is clear that therapeutic agents targeting signal-transduction pathways up-regulated under hypoxia might exhibit antiangiogenic properties reflecting, at least in part, their capability of decreasing HIF-1 activity. As recently illustrated with a PI3K inhibitor, the inhibition of Akt activity has indeed been shown to down-regulate HIF1-α (and VEGF expression), and increase oxygenation within tumor xenografts (26). Similarly to the aforementioned anti-PI3K and anti-VEGF studies, it is tempting to speculate that inhibiting the SphK1/SIP signaling might increase tumor sensitivity to radiation and chemotherapy in relation to the broader concept of “normalization of tumor vessels” as tumor oxygenation is known to enhance response to chemotherapy and radiation (27). The normalization of tumor vasculature initiated by anti-VEGF strategies relies on the reduction of vascular permeability (27). Interestingly, despite the fact that SIP has originally been shown to promote endothelial cell integrity (28, 29), it has recently been shown that SIP could increase vascular permeability (30) similar to VEGF; the canonical vascular permeability factor. Although it remains to be shown whether or not anti-SIP strategies might directly impact vascular permeability, it has been recently established that targeting SIP by using anti-SIP antibody could reduce plasma levels of VEGF in xenograft experimental models (31). Hence, it is conceivable that anti-SphK1/SIP strategies might, at least indirectly, by reducing VEGF levels, decrease vascular permeability, and thus, lead to normalization of tumor vessels.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 2/2/09; revised 3/2/09; accepted 3/3/09; published OnlineFirst 4/21/09.

Grant support: Centre National de la Recherche Scientifique (CNRS), Institut National du Cancer (INCa), La Ligue Contre le Cancer, Association pour la Recherche sur le Cancer (ARC), Association pour la Recherche sur les Tumeurs de la Prostate (ARTP), Association Française d’Urologie (AFU).
Cancer Res 2009; 69: (9). May 1, 2009

References


