Molecular Pharmacology Fast Forward. Published on September 25, 2006 as doi:10.1124/mol.106.031062
This article has not been copyedited and formatted. The final version may differ from this version.

MOL 31062

Baffled by Bafilomycin: An Anti-cancer Agent that Induces HIF-1α Expression

Gregg L. Semenza

Vascular Biology Program, Institute for Cell Engineering; Departments of Pediatrics, Medicine, Oncology, and Radiation Oncology; and McKusick-Nathans Institute of Genetic Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland

Molecular Pharmacology Fast Forward. Published on September 25, 2006 as DOI: 10.1124/mol.106.031062
This article has not been copyedited and formatted. The final version may differ from this version.

ABBREVIATIONS: HIF-1, hypoxia-inducible factor 1; EPO, erythropoietin; VHL, von Hippel-Lindau protein; VEGF, vascular endothelial growth factor; SDF1, stromal-derived factor 1; GLUT1, glucose transporter 1; ENO1, enolase 1; PGK1, phosphoglycerate kinase 1; IGF2, insulin-like growth factor 2; TGFA, transforming growth factor α; CBP, CREB binding protein; CREB, cAMP response element binding protein.

Molecular Pharmacology Fast Forward. Published on September 25, 2006 as DOI: 10.1124/mol.106.031062
This article has not been copyedited and formatted. The final version may differ from this version.

ABSTRACT

In an article presented in this issue of *Molecular Pharmacology*, Lim et al. investigate the anti-cancer effect of bafilomycin, an inhibitor of the vacuolar ATPase. The authors report that bafilomycin inhibits cell cycle progression and tumor growth by inducing the expression of hypoxia-inducible factor (HIF) 1α and the cyclin-dependent kinase inhibitor $p21^{CIP1}$, a surprising result because HIF-1α overexpression is associated with tumor growth and angiogenesis in preclinical models and with increased patient mortality in clinical studies. However, the authors demonstrate that bafilomycin-induced HIF-1α expression leads to increased *CIP1* gene expression but does not lead to increased expression of other HIF-1-regulated genes that promote tumor progression.

Molecular Pharmacology Fast Forward. Published on September 25, 2006 as DOI: 10.1124/mol.106.031062
This article has not been copyedited and formatted. The final version may differ from this version.

Rapid progress has been made in delineating the molecular mechanisms that underlie oncogenesis. Most recently, this has included the generation of a comprehensive compendium of the genes that are most commonly mutated in breast and colon cancers (Sjoblom et al., 2006). Many of these mutations promote cancer formation by dysregulating one of a limited number of key signaling pathways (Parsons et al., 2005; Vogelstein and Kinzler, 2004). In addition to genetic and epigenetic (Baylin and Chen, 2005) modifications of the cancer cell genome, responses to the tumor microenvironment play a critical role. In particular, the adaptation of tumor cells to hypoxia (Kaufman et al., 2004) represents a major selective force (Graeber et al., 1996) in the clonal evolution of human cancers (Nowell, 1976; Semenza, 2000).

A key transcriptional regulator that mediates adaptive responses of cancer cells to reduced O_2 availability is hypoxia-inducible factor 1 (HIF-1). HIF-1 is a heterodimer composed of a constitutively expressed HIF-1β subunit and an O_2-regulated HIF-1α subunit (Wang and Semenza, 1995; Wang et al., 1995). HIF-1 was originally identified as a transcriptional activator of the *EPO* gene, which encodes the protein that controls red blood cell production, and thus, blood O_2-carrying capacity (Semenza and Wang, 1992). Dozens of genes are now known to be transcriptionally activated by direct binding of HIF-1 under hypoxic conditions (Wenger et al., 2005; Hirota and Semenza, 2006). Microarray analyses indicate that the expression of hundreds of genes is activated or repressed by HIF-1 (Elvidge et al., 2006; Manalo et al., 2005).

Elevated HIF-1α protein levels are observed in the majority of human cancers, either as a direct result of intratumoral hypoxia or secondary to genetic alterations in oncogenes or tumor suppressor genes, and are associated with increased patient mortality (Semenza, 2003). The most dramatic example of tumor suppressor loss-of-function resulting in HIF-1 gain-of-function involves the von Hippel-Lindau (VHL) protein, which binds to HIF-1α under normoxic conditions and targets the protein for ubiquitination and proteasomal degradation (Maxwell et al.,

Molecular Pharmacology Fast Forward. Published on September 25, 2006 as DOI: 10.1124/mol.106.031062
This article has not been copyedited and formatted. The final version may differ from this version.

1999). VHL loss-of-function is observed in most sporadic renal cell carcinomas of the clear cell type and in all renal cancers in patients with the hereditary von Hippel-Lindau syndrome (reviewed in Kim and Kaelin, 2004). In these latter tumors, activation of HIF-1 transcriptional activity is the earliest identifiable sign of cellular transformation (Mandriota et al. 2002).

HIF-1 activates the transcription of target genes that encode proteins with critical roles in key aspects of cancer biology: *VEGF* and *SDF1* stimulate tumor angiogenesis; *GLUT1, ENO1*, and *PGK1* stimulate glucose transport and aerobic glycolysis; *EPO, IGF2*, and *TGFA* participate in autocrine growth factor signaling; and multidrug transporters *ABCB1* and *ABCG2* promote chemotherapy resistance (Hirota and Semenza, 2006). In addition, HIF-1 activates the transcription of genes encoding transcriptional repressors that extinguish the expression of E-cadherin, an event that is required for invasion and metastasis of cancers derived from epithelial cell types (Krishnamachary et al., 2006).

HIF-1α may also promote genomic instability by transcriptional repression of the *MSH2* and *MSH6* genes, which encode subunits of the mismatch repair enzyme MutSα, through a novel mechanism in which HIF-1α interacts, in the absence of HIF-1β, with promoter-bound Sp1 and functions as a co-repressor (Koshiji et al., 2005). This mechanism is similar to the role of HIF-1α in regulating the *CIP1* gene, which encodes the cyclin-dependent kinase inhibitor $p21^{CIP1}$ (Koshiji et al., 2004). In both cases, HIF-1α was shown to displace C-MYC from the promoter. In the case of the *MSH2* and *MSH6* genes, because C-MYC binding activates transcription, its displacement by HIF-1α results in transcriptional repression. In contrast, C-MYC binding represses *CIP1* transcription and its displacement results in de-repression, an effect that does not require either the DNA binding or transactivation functions of HIF-1α.

Given the important role of HIF-1 in cancer biology, there is great interest in identifying inhibitors of HIF-1 and many novel anti-cancer agents appear to act in part by inhibiting HIF-1

Molecular Pharmacology Fast Forward. Published on September 25, 2006 as DOI: 10.1124/mol.106.031062
This article has not been copyedited and formatted. The final version may differ from this version.

(Melillo, 2006; Semenza, 2006). Thus, Lim et al. (2006) investigated whether modulation of HIF-1 activity might contribute to the anti-cancer effects of the macrolide antibiotics bafilomycin A and concanamycin A. Surprisingly, they found increased HIF-1α levels following exposure of cancer cells to these compounds, which are known inhibitors of the vacuolar ATPase that is present in membranes of endoplasmic reticulum, Golgi, and vacuoles, where it generates an electrochemical gradient for protons that is required for metabolite transport and pH regulation (Bowman et al., 2006). Lim et al. (2006) showed that bafilomycin treatment increased the half-life of HIF-1α protein by inhibiting its interaction with VHL, an effect that was independent of any pH changes (Fig. 1). Further studies are required to determine whether the induction of HIF-1α by bafilomycin can be phenocopied by RNA interference targeting the vacuolar ATPase.

In a further surprise, the increased HIF-1α protein levels were not associated with increased transcription of HIF-1 target genes such as *VEGF*, *PGK1*, and *ENO1*. However, *CIP1* gene transcription was induced by bafilomycin, an effect that was associated with increased binding of HIF-1α and decreased binding of C-MYC to the *CIP1* promoter. Low nanomolar concentrations of bafilomycin induced cell cycle arrest in wild-type cells but not in cells that lacked expression of $p21^{CIP1}$ or HIF-1α. Injection of bafilomycin into mice bearing wild type or HIF-1α-null fibrosarcomas inhibited growth of the former but not the latter tumors. These results provide convincing evidence that the mechanism by which bafilomycin inhibits tumor growth is HIF-1α- and $p21^{CIP1}$-dependent (Figure 1).

What are the clinical implications of this fascinating study? Among the hundreds of genes that are activated by HIF-1, many promote tumor progression whereas others (such as *CIP1*) have the opposite effect. Since each tumor cell expresses a distinct subset of HIF-1-regulated genes, the net effect of increased HIF-1α expression cannot be predicted with certainty because it depends on which genes are expressed and what other genetic alterations are present

Molecular Pharmacology Fast Forward. Published on September 25, 2006 as DOI: 10.1124/mol.106.031062
This article has not been copyedited and formatted. The final version may differ from this version.

within the cell (*e.g.* loss-of-function mutations in the *CIP1* coding sequence that would render its transcriptional regulation irrelevant). Koshiji et al. (2004, 2005) demonstrated that HIF-1α can function as a transcriptional co-factor independent of its dimerization with HIF-1β to form a DNA binding protein. The data of Lim et al. (2006) suggest that the functions of HIF-1α that are HIF-1β-dependent and HIF-1β-independent may be pharmacologically separable. Further studies are required to prove that the effect of bafilomycin on cell cycle progression and $p21^{CIP1}$ expression is HIF-1β-independent. In addition, it will be interesting to determine whether failure of HIF-1α to dimerize with HIF-1β or to recruit the co-activators p300 and CBP underlies the lack of HIF-1 transcriptional activity in bafilomycin-treated cells under non-hypoxic conditions.

Based on their results, the authors speculate that bafilomycin may be a useful therapeutic agent. Their data also suggest that in cancer cells in which HIF-1α overexpression is driving $p21^{CIP1}$ expression, administration of compounds that decrease HIF-1α expression (Melillo, 2006; Semenza, 2006) may cause these cells to re-enter the cell cycle and thereby increase their sensitivity to standard chemotherapy. The intriguing results presented by Lim et al. (2006) underscore the complex regulatory networks that are controlled by HIF-1 in cancer cells and the tremendous challenges associated with the clinical translation of molecular pharmacology.

References

Baylin SB and Chen WY (2005) Aberrant gene silencing in tumor progression: implications for control of cancer. *Cold Spring Harb Symp Quant Biol* **70**:427-433.

Bowman BJ, McCall ME, Baertsch R, and Bowman EJ (2006) A model for the proteolipid ring and bafilomycin/concanamycin binding site in the vacuolar ATPase of Neurospora crassa. *J Biol Chem* Aug 15; [Epub ahead of print].

Elvidge GP, Glenny L, Appelhoff RJ, Ratcliffe PJ, Ragoussis J, and Gleadle JM (2006) Concordant regulation of gene expression by hypoxia and 2-oxoglutarate-dependent dioxygenase inhibition: the role of HIF-1α, HIF-2α, and other pathways. *J Biol Chem* **281**:15215-15226.

Graeber TG, Osmanian C, Jacks T, Housman DE, Koch CJ, Lowe SW, and Giaccia AJ (1996) Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. *Nature* **379**:88-91.

Hirota K and Semenza GL (2006) Regulation of angiogenesis by hypoxia-inducible factor 1. *Crit Rev Oncol Hematol* **59**:15-26.

Kaufman B, Scharf O, Arbeit J, Ashcroft M, Brown JM, Bruick RK, Chapman JD, Evans SM, Giaccia AJ, Harris AL, et al. (2004) Proceedings of the Oxygen Homeostasis/Hypoxia Meeting. *Cancer Res* **64**:3350-3356.

Kim WY and Kaelin WG (2004) Role of VHL gene mutation in human cancer. *J Clin Oncol* **22**:4991-5004.

Koshiji M, Kageyama Y, Pete EA, Horikawa I, Barrett JC, and Huang LE (2004) HIF-1α induces cell cycle arrest by functionally counteracting Myc. *EMBO J* **23**:1949-1956.

Koshiji M, To KK, Hammer S, Kumamoto K, Harris AL, Modrich P, and Huang LE (2005) HIF-1α induces genetic instability by transcriptionally downregulating MutSα expression. *Mol Cell* **17**:793-803.

Krishnamachary B, Zagzag D, Nagasawa H, Rainey K, Okuyama H, Baek JH, and Semenza GL (2006) Hypoxia-inducible factor-1-dependent repression of E-cadherin in von Hippel-Lindau tumor suppressor-null renal cell carcinoma mediated by TCF3, ZFHX1A, and ZFHX1B. *Cancer Res* **66**:2725-2731.

Lim JH, Park JW, Kim MS, Park SK, Johnson RS, and Chun YS (2006) Bafilomycin induces the p21-mediated growth inhibition of cancer cells under hypoxic conditions by expressing HIF-1α. *Mol Pharmacol* Aug 28; [Epub ahead of print].

Manalo DJ, Rowan A, Lavoie T, Natarajan L, Kelly BD, Ye SQ, Garcia JG, and Semenza GL (2005) Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. *Blood* **105**:659-669.

Mandriota SJ, Turner KJ, Davies DR, Murray PG, Morgan NV, Sowter HM, Wykoff CC, Maher ER, Harris AL, Ratcliffe PJ, et al. (2002) HIF activation identifies early lesions in VHL kidneys: evidence for site-specific tumor suppressor function in the nephron. *Cancer Cell* **1**:459-468.

Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER, et al. (1999) The tumor suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* **399**:271-275.

Melillo G (2006) Inhibiting hypoxia-inducible factor 1 for cancer therapy. *Mol Cancer Res* **4**:601-605.

Nowell PC (1976) The clonal evolution of tumor cell populations. *Science (Wash DC)* **194**:23-28.

Parsons DW, Wang TL, Samuels Y, Bardelli A, Cummins JM, DeLong L, Silliman N, Ptak J, Szabo S, Willson JK, et al. (2005) Colorectal cancer: mutations in a signalling pathway. *Nature* **436**:792.

Semenza GL (2000) Hypoxia, clonal selection, and the role of HIF-1 in tumor progression. *Crit Rev Biochem Mol Biol* **35**:71-103.

Semenza GL (2003) Targeting HIF-1 for cancer therapy. *Nat Rev Cancer* **3**:721-732.

Semenza GL (2006) Development of novel therapeutic strategies that target HIF-1. *Expert Opin Ther Targets* **10**:267-280.

Semenza GL and Wang GL (1992) 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* **12**:5447-5454.

Sjoblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber T, Mandelker D, Leary RJ, Ptak J, Silliman N, et al. (2006) The consensus coding sequences of human breast and colorectal cancers. *Science (Wash DC)* Sep 7; [Epub ahead of print].

Vogelstein B and Kinzler KW (2004) Cancer genes and the pathways they control. *Nat Med* **10**:789-799.

Wang GL, Jiang BH, Rue EA, and Semenza GL (1995) Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. *Proc Natl Acad Sci USA* **92**:5510-5514.

Wang GL and Semenza GL (1995) Purification and characterization of hypoxia-inducible factor 1. *J Biol Chem* **270**:1230-1237.

Wenger RH, Stiehl DP, and Camenisch G (2005) Integration of oxygen signaling at the consensus HRE. *Sci STKE* **306**:re12.

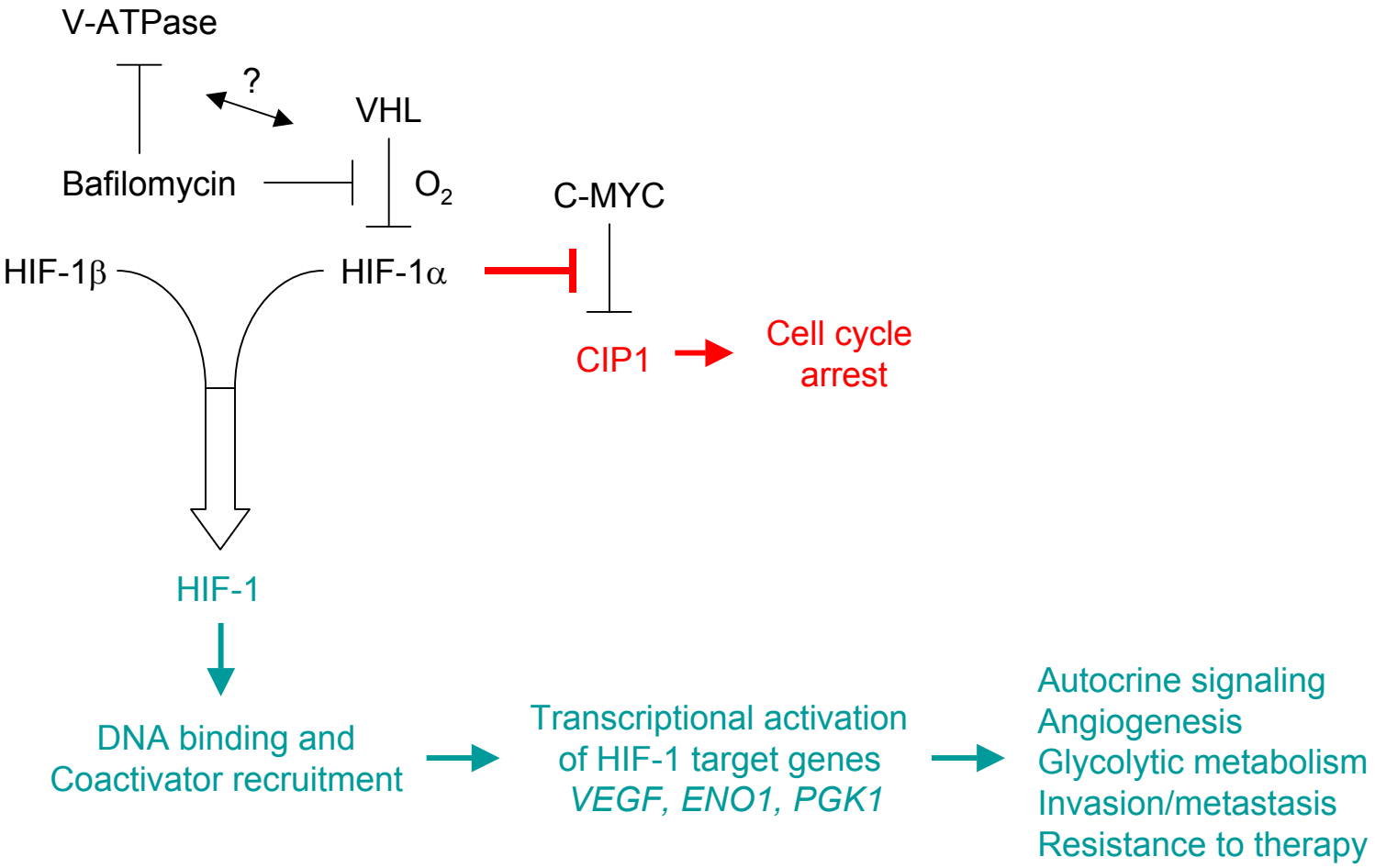
Footnotes

Work in the author's laboratory is supported by grants from the Flight Attendants' Medical Research Institute, National Cancer Institute, National Heart Lung Blood Institute, National Institute of Aging, National Institute of General Medical Sciences, and the American Diabetes Association.

Molecular Pharmacology Fast Forward. Published on September 25, 2006 as DOI: 10.1124/mol.106.031062
This article has not been copyedited and formatted. The final version may differ from this version.

Figure Legend

Fig. 1. Bafilomycin induces HIF-1α-mediated de-repression of *CIP1* gene transcription and cell cycle arrest. Bafilomycin also inhibits the vacuolar ATPase (V-ATPase) but it is not clear whether this effect is required for its induction of HIF-1α expression. Despite increased HIF-1α expression, HIF-1-dependent gene transcription (which requires HIF-1α:HIF-1β dimerization and the recruitment of coactivators) is not induced by bafilomycin.


V-ATPase
?
VHL
Bafilomycin
O_2
C-MYC
HIF-1β
HIF-1α
CIP1
Cell cycle arrest
HIF-1
DNA binding and Coactivator recruitment
Transcriptional activation of HIF-1 target genes *VEGF, ENO1, PGK1*
Autocrine signaling
Angiogenesis
Glycolytic metabolism
Invasion/metastasis
Resistance to therapy