Published OnlineFirst August 28, 2006; DOI: 10.1158/1541-7786.MCR-06-0235

Subject Review

Inhibiting Hypoxia-Inducible Factor 1 for Cancer Therapy

Giovanni Melillo

Developmental Therapeutics Program, Science Applications International Corporation-Frederick, Inc., National Cancer Institute, Frederick, Maryland

Abstract

Hypoxia has long been recognized as a common feature of solid tumors and a negative prognostic factor for response to treatment and survival of cancer patients. The discovery of hypoxia-inducible factor 1 (HIF-1), a molecular determinant of the response of mammalian cells to hypoxia, has led to the identification of a ''molecular target'' of hypoxia suitable for the development of cancer therapeutics. Early controversy about whether or not HIF-1 is a good target for therapy has not discouraged academic groups and pharmaceutical companies from actively engaging in the discovery of small-molecule inhibitors of HIF. However, what is the best strategy to inhibit HIF and how HIF inhibitors should be developed for treatment of human cancers is still poorly defined. In this review, aspects related to the identification and early development of novel HIF inhibitors are discussed. Identification and validation of pharmacodynamic end points relevant to the HIF-1 pathway is essential for a rational development of HIF inhibitors. Integration of these biomarkers in early clinical trials may provide valuable information to determine the contribution of HIF inhibitors to response to therapy. Finally, HIF inhibitors should be incorporated in combination strategies to effectively target multiple cellular components of the tumor microenvironment and redundant signaling pathways frequently deregulated in human cancer. (Mol Cancer Res 2006;4(9):601–5)

Over the last few years, a better appreciation of the role that the tumor microenvironment plays in tumor progression has been associated with a paradigm shift in how to best develop therapeutic strategies for cancer treatment (1). As a consequence, renewed emphasis has also been placed on key features of the tumor microenvironment such as hypoxia. Indeed, it has long been recognized that hypoxia contributes to selecting cancer cells resistant to apoptosis and mediates resistance to chemotherapy and radiation therapy (2, 3). Nevertheless, it has not been until the discovery of the transcription factor hypoxia-inducible factor 1 (HIF-1) that a ''molecular target'' underlying the presence of hypoxia in solid tumors has been identified and proposed for development of cancer therapeutics (4-6).

HIF-1 is a master regulator of the transcriptional response of mammalian cells to oxygen deprivation. Since its discovery in the early 1990s, HIF-1 has rapidly attracted interest both for its involvement in fundamental biological processes, including but not limited to tumor metabolism, angiogenesis, metastasis, and inflammation, and for its potential role as therapeutic target (4-6). However, the complexity of the role that HIF-1 may play in cancer biology, its dual role in promoting cell survival and inducing apoptosis, and the presence of two HIF-α subunits (HIF-1α and HIF-2α), which may have unique and specific roles in different tumor types, have contributed to generating some early controversy about the role of HIF-1 as therapeutic target. Whether or not HIF-1 is a good target for development of cancer therapeutics has been addressed in a number of experimental models, which have led, not unexpectedly, to somewhat controversial results. Indeed, different results have been reported depending on the cell type used (7-9), the HIF-α subunit targeted (10-12), the site of tumor injection (e.g., s.c. versus orthotopic; refs. 13, 14), and the timing of HIF-1α inhibition, more active in early rather than late tumor growth (15). Because HIF-1 has not been directly implicated in oncogenic transformation, although evidence has been provided that it can cooperate with AKT in melanomagenesis (16), it has been difficult to translate results from *in vitro* studies to animal models. Such controversial results reflect, at least in part, the complexity of the involvement of HIF-1 in human cancers and the known limitations of experimental animal models. At least two conclusions can be drawn from these studies. The first is that the contribution of HIF-1 to tumor progression is highly cell type and context dependent, affected by the genetic complexity of the cell lines studied and influenced by the stromal component, which is neither properly represented in the xenograft models nor affected by genetic manipulation of HIF in cancer cells. The second conclusion is that manipulation of HIF-1 activity outside of the appropriate genetic context may actually generate misleading conclusions, as clonal expansion of HIF overexpressing cells, which occurs in human cancers, requires the temporal selection of specific genetic alterations and conducive environmental conditions.

Received 7/31/06; accepted 8/9/06.
Grant support: Federal funds from the National Cancer Institute, NIH, contract N01-CO-12400, and the Developmental Therapeutics Program, Division of Cancer Treatment and Cancer Diagnosis, National Cancer Institute.
Note: The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.
Requests for reprints: Giovanni Melillo, Developmental Therapeutics Program-Tumor Hypoxia Laboratory, National Cancer Institute, Room 218, Building 432, Frederick, MD 21702. Phone: 301-846-5050; Fax: 301-846-6081. E-mail: melillog@ncifcrf.gov

doi:10.1158/1541-7786.MCR-06-0235

Published OnlineFirst August 28, 2006; DOI: 10.1158/1541-7786.MCR-06-0235

How then can results of preclinical studies be translated to human cancer? In contrast to the results obtained in animal models, the studies conducted in cancer patients have been overall more consistent with a positive role of HIF in tumor progression. In fact, overwhelming evidence indicates that HIF-α is indeed overexpressed in the majority of human cancers (17-19), where it is associated with patient mortality and poor response to treatment (20-24), findings that can be hardly reconciled with a negative role of HIF-1 in tumor growth. Importantly, the association of several oncogenic and receptor tyrosine kinase–dependent signaling pathways, frequently deregulated in human cancers, with HIF-1α activation also suggests that many of these pathways converge on or implicate HIF-1 in mediating cell survival and growth (25-28). However, staining of HIF-1α in human cancers is variable, from few positive cells up to the majority of tumor tissue, and characterized by distinct patterns of expression, of which the implications and biological consequences are still poorly understood. HIF-1α expression in solid tumors has been detected in hypoxic perinecrotic areas, in stromal inflammatory cells, as well as in patterns unrelated to tissue oxygen concentrations, most likely dependent on genetic alterations and dysregulation of growth factors signaling (17-19). Then, rather than asking the question about whether or not HIF-1 should be inhibited, we ought to address the issue of how to do it. Most of the efforts to identify HIF inhibitors have converged on the discovery of small molecules, both in academic centers and pharmaceutical companies, despite the skepticism associated with targeting transcription factors. The identification of selective HIF-1 inhibitors would not only be useful for the potential therapeutic implications but also for their application as analytic tools to further define the role of HIF in human cancers. Earlier efforts in this area have been associated with hope that universal and specific inhibitors of HIF might be identified. Perhaps not unexpectedly, only a few examples of HIF inhibitors that potentially target selective pathways associated with HIF activation have been described, among which echinomycin (29) and synthetic polyamides (30) that inhibit HIF-1 DNA binding, and chetomin (31), which blocks recruitment of coactivator p300/CBP (Fig. 1). In addition, ongoing efforts in targeting dimerization of HIF-α with HIF-1β might lead to unanticipated results (32, 33), given the recent apparent success in the identification of small-molecule inhibitors of protein-protein interaction. However, none of these agents has been thus far proposed for clinical development. In contrast, a growing list of nonselective HIF-1 inhibitors has been generated over the last few years (34, 35). In fact, most of the described HIF-1 inhibitors do so by altering signal transduction pathways that are indirectly associated with HIF or that are part of more complex pathways relevant to human cancer, clearly limiting the specificity of their activity. Small-molecule inhibitors of HIF have been identified that target chaperone proteins (36, 37), microtubules (38), topoisomerase I (39), soluble guanylate-cyclase (40), and thioredoxin (41), as well as a number of signaling pathways that have been associated with HIF-1α activation including mammalian target of rapamycin (26, 28), AKT (42), Her2/Neu

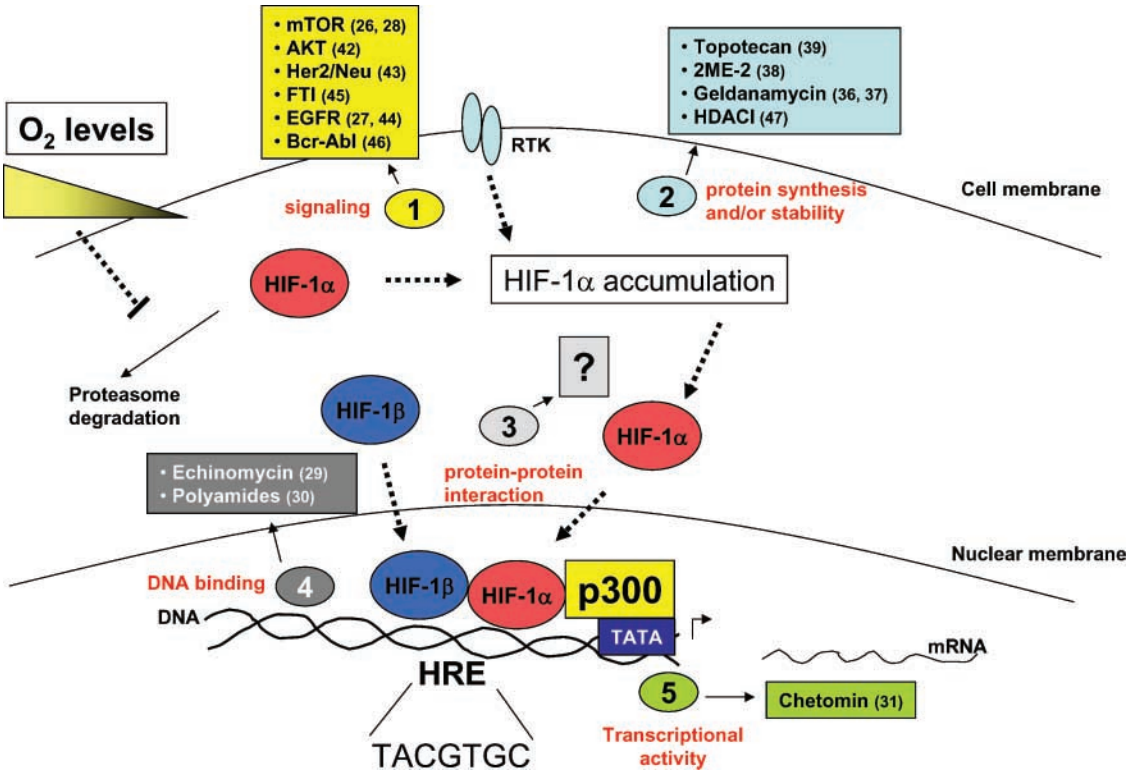

FIGURE 1. Possible targets of small-molecule inhibitors of HIF-1.

Published OnlineFirst August 28, 2006; DOI: 10.1158/1541-7786.MCR-06-0235

(43), epidermal growth factor receptor (27, 44), farnesyltransferase (45), Bcr-Abl (46), and histone deacetylase (ref. 47; Fig. 1). On the one hand, the involvement of HIF-1α in such a broad range of signaling pathways emphasizes its potential role as downstream "effector" molecule on which multiple pathways may converge; on the other hand, it raises the question about how to determine the contribution of HIF-1 inhibition to tumor growth in each individual case.

Then, how should nonselective HIF inhibitors be developed for treatment of human cancers? The fact that most HIF inhibitors described thus far are not selective does not necessarily rule out that these agents might be used in the clinic to target HIF. In fact, several of these agents, including 17-*N*-allylamino-17-demethoxygeldanamycin, 2-methoxyestradiol, and small molecules targeting receptor tyrosine kinase–dependent signaling pathways, are currently in clinical development for cancer therapy (48). Although for some of these agents (e.g., receptor tyrosine kinase inhibitors) HIF may not become the primary target of their clinical application, the recurrent implication of HIF-1 in receptor tyrosine kinase–dependent signaling pathways would suggest that HIF-1 inhibition might become a "biomarker" of biological activity. However, because most of the HIF inhibitors described affect multiple signaling pathways and/or targets indirectly associated with HIF, assessment of their activity as HIF inhibitors cannot be based on therapeutic efficacy, which might be unrelated to HIF inhibition. Instead, the preclinical development of HIF inhibitors should be guided by evidence in animal models that the target is modulated and that this inhibition is associated with meaningful biological consequences. Several approaches have been used in this regard, ranging from noninvasive imaging of hypoxia response element–dependent expression of bioluminescence (49) to dynamic contrast imaging techniques (50), to assessment of tissue molecular end points relevant to the HIF pathway (e.g., HIF-1α expression assessed by immunohistochenistry, HIF-target genes; refs. 49, 51). The identification and validation of pharmacodynamic end points associated with and relevant to HIF inhibition is also essential for early clinical development of HIF inhibitors, where tumor shrinkage alone cannot be expected to be a reliable determinant of activity on the HIF pathway. Early clinical trials of HIF inhibitors should then be designed to achieve (*a*) evidence of HIF inhibition in cancer tissue and (*b*) protracted schedules of administration in the absence of undesired or unmanageable adverse effects. Unlike cytotoxic agents that are given for short courses at the maximum tolerated dose to capitalize on killing of cancer cells, HIF-targeted therapeutics should be aimed at achieving sustained HIF inhibition. This conclusion, consistent with the current use of signaling targeting agents in cancer therapy, is also suggested by recent studies in which protracted, but not intermittent, administration of topotecan, a topoisomerase I poison that inhibits HIF-1, was associated with inhibition of tumor angiogenesis and cancer growth in xenograft models (49). A number of questions that have yet to be convincingly answered in preclinical models hamper the current development of novel HIF inhibitors. Is a HIF inhibitor expected to be active as single agent? Which are meaningful downstream end points that are associated with HIF inhibition? Are any of the available imaging techniques useful to assess the activity of HIF inhibitors? How should patients, in whom to test these agents, be selected? Should HIF expression be required for treatment or might HIF expression predict response to therapy? In an attempt to answer some of these questions, a clinical trial is being conducted at the National Cancer Institute (Bethesda, MD) in patients whose cancers express HIF-1α in more than 10% of tumor tissue.[1] Patients are treated with oral topotecan on a daily ×5 days ×2 weeks schedule every 28 days at a dose below the predicted maximum tolerated dose. Patients are selected based on tissue expression of HIF-1α, and the primary end point of the study is inhibition of HIF-1α expression, assessed by immunohistochemistry, in tumor tissue. A number of correlative studies, including imaging with 18F-fluorodeoxyglucose-positron emission tomography and dynamic contrast-enhanced magnetic resonance imaging to assess tumor metabolism and blood flow, respectively, mRNA expression of HIF-target genes, and soluble markers of angiogenesis, are also conducted to validate potential effects on the primary target. This clinical trial may provide useful information about the feasibility of such studies and the downstream biological effects associated with modulation of HIF-1 activity in tumor tissue.

Finally, two aspects relevant to the development of HIF inhibitors should be considered. The first is that HIF-1α expression is frequently detected in the inflammatory infiltrate of solid tumors, a feature poorly represented in animal models (52). Although the regulation of HIF-1 in stromal infiltrating cells is still poorly understood, recent evidence has shown that HIF-1α plays a critical role in myeloid cell (53) as well as endothelial cell (54, 55) functions. The effect of small-molecule inhibitors of HIF-1 on the cellular components of the tumor microenvironment has not been adequately addressed in preclinical models, and modulation of HIF activity in these cells may be instrumental for therapeutic success. The second aspect to consider is that HIF-1 inhibition alone may not be sufficient to halt angiogenesis and tumor growth, as HIF-independent pathways may bypass or overcome HIF inhibition (56). Thus, combination of HIF inhibitors with conventional therapies or emerging molecular targeted agents may be required and warranted. Combination therapies may be indicated not only for the potential synergistic inhibition of multiple and redundant signaling pathways, both HIF dependent and HIF independent, but also for the potential involvement of HIF in mediating resistance to radiation therapy (57) and chemotherapy (58, 59). The combination of HIF inhibitors with conventional therapeutic strategies and novel molecular targeted agents is also essential to finally integrate hypoxia-targeting approaches with standard therapeutics tools available to oncologists.

Acknowledgments

I thank Robert H. Shoemaker, John Cardellina, and all the members of my laboratory for helpful and inspiring discussions, and Drs. A.J. Murgo, M. Gutierrez, S. Kummar, A. Chen, J.H. Doroshow, and M. Raffeld for their help and support in the conduction of the clinical trial.

[1] http://www.clinicaltrials.gov/ct/show/NCT00117013?order=10.

Published OnlineFirst August 28, 2006; DOI: 10.1158/1541-7786.MCR-06-0235

References

1. Melillo G, Semenza GL. Meeting report: exploiting the tumor microenvironment for therapeutics. Cancer Res 2006;66:4558–60.

2. Brown JM, Giaccia AJ. The unique physiology of solid tumors: opportunities (and problems) for cancer therapy. Cancer Res 1998;58:1408–16.

3. Harris AL. Hypoxia—a key regulatory factor in tumour growth. Nat Rev Cancer 2002;2:38–47.

4. Semenza GL. Targeting HIF-1 for cancer therapy. Nat Rev Cancer 2003;3:721–32.

5. Melillo G. HIF-1: a target for cancer, ischemia and inflammation-too good to be true? Cell Cycle 2004;3:154–5.

6. Giaccia A, Siim BG, Johnson RS. HIF-1 as a target for drug development. Nat Rev Drug Discov 2003;2:803–11.

7. Maxwell PH, Dachs GU, Gleadle JM, et al. Hypoxia-inducible factor-1 modulates gene expression in solid tumors and influences both angiogenesis and tumor growth. Proc Natl Acad Sci U S A 1997;94:8104–9.

8. Ryan HE, Poloni M, McNulty W, et al. Hypoxia-inducible factor-1α is a positive factor in solid tumor growth. Cancer Res 2000;60:4010–5.

9. 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.

10. Covello KL, Simon MC, Keith B. Targeted replacement of hypoxia-inducible factor-1α by a hypoxia-inducible factor-2α knock-in allele promotes tumor growth. Cancer Res 2005;65:2277–86.

11. Kondo K, Klco J, Nakamura E, Lechpammer M, Kaelin WG, Jr. Inhibition of HIF is necessary for tumor suppression by the von Hippel-Lindau protein. Cancer Cell 2002;1:237–46.

12. Acker T, Diez-Juan A, Aragones J, et al. Genetic evidence for a tumor suppressor role of HIF-2α. Cancer Cell 2005;8:131–41.

13. Blouw B, Song H, Tihan T, et al. The hypoxic response of tumors is dependent on their microenvironment. Cancer Cell 2003;4:133–46.

14. Stoeltzing O, McCarty MF, Wey JS, et al. Role of hypoxia-inducible factor 1α in gastric cancer cell growth, angiogenesis, and vessel maturation. J Natl Cancer Inst 2004;96:946–56.

15. Li L, Lin X, Staver M, et al. Evaluating hypoxia-inducible factor-1α as a cancer therapeutic target via inducible RNA interference *in vivo*. Cancer Res 2005;65:7249–58.

16. Bedogni B, Welford SM, Cassarino DS, Nickoloff BJ, Giaccia AJ, Powell MB. The hypoxic microenvironment of the skin contributes to Akt-mediated melanocyte transformation. Cancer Cell 2005;8:443–54.

17. Zhong H, De Marzo AM, Laughner E, et al. Overexpression of hypoxia-inducible factor 1α in common human cancers and their metastases. Cancer Res 1999;59:5830–5.

18. Talks KL, Turley H, Gatter KC, et al. The expression and distribution of the hypoxia-inducible factors HIF-1α and HIF-2α in normal human tissues, cancers, and tumor-associated macrophages. Am J Pathol 2000;157:411–21.

19. Bos R, Zhong H, Hanrahan CF, et al. Levels of hypoxia-inducible factor-1 α during breast carcinogenesis. J Natl Cancer Inst 2001;93:309–14.

20. Aebersold DM, Burri P, Beer KT, et al. Expression of hypoxia-inducible factor-1α: a novel predictive and prognostic parameter in the radiotherapy of oropharyngeal cancer. Cancer Res 2001;61:2911–6.

21. Birner P, Schindl M, Obermair A, Breitenecker G, Oberhuber G. Expression of hypoxia-inducible factor 1α in epithelial ovarian tumors: its impact on prognosis and on response to chemotherapy. Clin Cancer Res 2001;7:1661–8.

22. Birner P, Schindl M, Obermair A, Plank C, Breitenecker G, Oberhuber G. Overexpression of hypoxia-inducible factor 1α is a marker for an unfavorable prognosis in early-stage invasive cervical cancer. Cancer Res 2000;60:4693–6.

23. Bos R, van der, GP, Greijer AE, et al. Levels of hypoxia-inducible factor-1α independently predict prognosis in patients with lymph node negative breast carcinoma. Cancer 2003;97:1573–81.

24. Koukourakis MI, Bentzen SM, Giatromanolaki A, et al. Endogenous markers of two separate hypoxia response pathways (hypoxia inducible factor 2 α and carbonic anhydrase 9) are associated with radiotherapy failure in head and neck cancer patients recruited in the CHART randomized trial. J Clin Oncol 2006;24:727–35.

25. Pouyssegur J, Dayan F, Mazure NM. Hypoxia signalling in cancer and approaches to enforce tumour regression. Nature 2006;441:437–43.

26. Majumder PK, Febbo PG, Bikoff R, et al. mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. Nat Med 2004;10:594–601.

27. Pore N, Jiang Z, Gupta A, Cerniglia G, Kao GD, Maity A. EGFR tyrosine kinase inhibitors decrease VEGF expression by both hypoxia-inducible factor (HIF)-1-independent and HIF-1-dependent mechanisms. Cancer Res 2006;66:3197–204.

28. Hudson CC, Liu M, Chiang GG, et al. Regulation of hypoxia-inducible factor 1α expression and function by the mammalian target of rapamycin. Mol Cell Biol 2002;22:7004–14.

29. Kong D, Park EJ, Stephen AG, et al. Echinomycin, a small-molecule inhibitor of hypoxia-inducible factor-1 DNA-binding activity. Cancer Res 2005;65:9047–55.

30. Olenyuk BZ, Zhang GJ, Klco JM, Nickols NG, Kaelin WG, Jr., Dervan PB. Inhibition of vascular endothelial growth factor with a sequence-specific hypoxia response element antagonist. Proc Natl Acad Sci U S A 2004;101:16768–73.

31. Kung AL, Zabludoff SD, France DS, et al. Small molecule blockade of transcriptional coactivation of the hypoxia-inducible factor pathway. Cancer Cell 2004;6:33–43.

32. Yang J, Zhang L, Erbel PJ, et al. Functions of the Per/ARNT/Sim domains of the hypoxia-inducible factor. J Biol Chem 2005;280:36047–54.

33. Park EJ, Kong D, Fisher R, Cardellina J, Shoemaker RH, Melillo G. Targeting the PAS-A Domain of HIF-1α for Development of Small Molecule Inhibitors of HIF-1. Cell Cycle 2006;5:e1–e7 [EPUB].

34. Rapisarda A, Uranchimeg B, Scudiero DA, et al. Identification of small molecule inhibitors of hypoxia-inducible factor 1 transcriptional activation pathway. Cancer Res 2002;62:4316–24.

35. Semenza GL. Development of novel therapeutic strategies that target HIF-1. Expert Opin Ther Targets 2006;10:267–80.

36. Mabjeesh NJ, Post DE, Willard MT, et al. Geldanamycin induces degradation of hypoxia-inducible factor 1α protein via the proteosome pathway in prostate cancer cells. Cancer Res 2002;62:2478–82.

37. Isaacs JS, Jung YJ, Mimnaugh EG, Martinez A, Cuttitta F, Neckers LM. Hsp90 regulates a von Hippel Lindau-independent hypoxia-inducible factor-1 α-degradative pathway. J Biol Chem 2002;277:29936–44.

38. Mabjeesh NJ, Escuin D, LaVallee TM, et al. 2ME2 inhibits tumor growth and angiogenesis by disrupting microtubules and dysregulating HIF. Cancer Cell 2003;3:363–75.

39. Rapisarda A, Uranchimeg B, Sordet O, Pommier Y, Shoemaker RH, Melillo G. Topoisomerase I-mediated inhibition of hypoxia-inducible factor 1: mechanism and therapeutic implications. Cancer Res 2004;64:1475–82.

40. Yeo EJ, Chun YS, Cho YS, et al. YC-1: a potential anticancer drug targeting hypoxia-inducible factor 1. J Natl Cancer Inst 2003;95:516–25.

41. Welsh SJ, Williams RR, Birmingham A, et al. The thioredoxin redox inhibitors 1-methylpropyl 2-imidazolyl disulfide and pleurotin inhibit hypoxia-induced factor 1α and vascular endothelial growth factor formation. Mol Cancer Ther 2003;2:235–43.

42. Tan C, de Noronha RG, Roecker AJ, et al. Identification of a novel small-molecule inhibitor of the hypoxia-inducible factor 1 pathway. Cancer Res 2005;65:605–12.

43. Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL. HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1α (HIF-1α) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. Mol Cell Biol 2001;21:3995–4004.

44. Zhong H, Chiles K, Feldser D, et al. Modulation of hypoxia-inducible factor 1α expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. Cancer Res 2000;60:1541–5.

45. Han JY, Oh SH, Morgillo F, et al. Hypoxia-inducible factor 1α and antiangiogenic activity of farnesyltransferase inhibitor SCH66336 in human aerodigestive tract cancer. J Natl Cancer Inst 2005;97:1272–86.

46. Mayerhofer M, Valent P, Sperr WR, Griffin JD, Sillaber C. BCR/ABL induces expression of vascular endothelial growth factor and its transcriptional activator, hypoxia inducible factor-1α, through a pathway involving phosphoinositide 3-kinase and the mammalian target of rapamycin. Blood 2002;100:3767–75.

47. Kong X, Lin Z, Liang D, Fath D, Sang N, Caro J. Histone deacetylase inhibitors induce VHL and ubiquitin-independent proteasomal degradation of hypoxia-inducible factor 1α. Mol Cell Biol 2006;26:2019–28.

48. Thomas GV, Tran C, Mellinghoff IK, et al. Hypoxia-inducible factor determines sensitivity to inhibitors of mTOR in kidney cancer. Nat Med 2006;12:122–7.

49. Rapisarda A, Zalek J, Hollingshead M, et al. Shoemaker RH, Melillo G, Schedule-dependent inhibition of hypoxia-inducible factor-1α protein accumulation, angiogenesis, and tumor growth by topotecan in U251-HRE glioblastoma xenografts. Cancer Res 2004;64:6845–8.

Published OnlineFirst August 28, 2006; DOI: 10.1158/1541-7786.MCR-06-0235

50. Jordan BF, Runquist M, Raghunand N, et al. Dynamic contrast-enhanced and diffusion MRI show rapid and dramatic changes in tumor microenvironment in response to inhibition of HIF-1α using PX-478. Neoplasia 2005;7:475–85.

51. Welsh S, Williams R, Kirkpatrick L, Paine-Murrieta G, Powis G. Antitumor activity and pharmacodynamic properties of PX-478, an inhibitor of hypoxia-inducible factor-1α. Mol Cancer Ther 2004;3:233–44.

52. Murdoch C, Giannoudis A, Lewis CE. Mechanisms regulating the recruitment of macrophages into hypoxic areas of tumors and other ischemic tissues. Blood 2004;104:2224–34.

53. Cramer T, Yamanishi Y, Clausen BE, et al. HIF-1α is essential for myeloid cell-mediated inflammation. Cell 2003;112:645–57.

54. Tang N, Wang L, Esko J, et al. Loss of HIF-1α in endothelial cells disrupts a hypoxia-driven VEGF autocrine loop necessary for tumorigenesis. Cancer Cell 2004;6:485–95.

55. Calvani M, Rapisarda A, Uranchimeg B, Shoemaker RH, Melillo G. Hypoxic induction of a HIF-1α-dependent bFGF autocrine loop drives angiogenesis in human endothelial cells. Blood 2005;107:2705–12.

56. Mizukami Y, Jo WS, Duerr EM, et al. Induction of interleukin-8 preserves the angiogenic response in HIF-1α-deficient colon cancer cells. Nat Med 2005;11:992–7.

57. Moeller BJ, Cao Y, Li CY, Dewhirst MW. Radiation activates HIF-1 to regulate vascular radiosensitivity in tumors: role of reoxygenation, free radicals, and stress granules. Cancer Cell 2004;5:429–41.

58. Unruh A, Ressel A, Mohamed HG, et al. The hypoxia-inducible factor-1 α is a negative factor for tumor therapy. Oncogene 2003;22:3213–20.

59. Brown LM, Cowen RL, Debray C, et al. Reversing hypoxic cell chemoresistance *in vitro* using genetic and small molecule approaches targeting hypoxia inducible factor-1. Mol Pharmacol 2005;69:411–8.

AACR American Association for Cancer Research

Molecular Cancer Research

Inhibiting Hypoxia-Inducible Factor 1 for Cancer Therapy

Giovanni Melillo

Mol Cancer Res 2006;4:601-605. Published OnlineFirst August 28, 2006.