Published in final edited form as:

Clin Cancer Res. 2009 October 1; 15(19): 5945–5946. doi:10.1158/1078-0432.CCR-09-1650.

Inhibiting the hypoxia response for cancer therapy – the new kid on the block

Mei Yee Koh¹, Taly R. Spivak-Kroizman¹, and Garth Powis²

Department of Experimental Therapeutics, M.D. Anderson Cancer Center, Houston, Texas, 77030

Summary

The HIF-1 α inhibitor KC7F2 described in this issue of *Clinical Cancer Research* is the newest addition to an emerging class of antitumor agents targeting the hypoxia response. Here we discuss the proposed mechanism of action of KC7F2 and its potential strengths and limitations in comparison with other promising HIF-1 α inhibitors.

In this issue of *Clinical Cancer Research*, Narita *et al.* describe the identification of a novel HIF-1 α inhibitor, KC7F2, using a HIF-reporter cell-based screen of a natural product-like chemical library¹. KC7F2 treatment is reported to decrease HIF-1 α levels in a variety of cancer cell lines independently of *p53* or *PTEN* status and to inhibit the activation of downstream HIF-1 target genes such as carbonic anhydrase IX, matrix metalloproteinase II, endothelin 1 and enolase 1. KC7F2 is shown to inhibit the proliferation of cancer cells, an effect that is increased in hypoxia, while non-tumor cells are less sensitive. The authors propose that KC7F2 decreases HIF-1 α levels by downregulating HIF-1 α protein synthesis. KC7F2 is the second HIF-1 α inhibitor described by the Van Meir group. The first, 103D5R, was reported to act similarly through inhibition of HIF-1 α translation².

Hypoxia or low oxygen tension is a feature common in all solid tumors. Tumor hypoxia is of major clinical significance since it can both promote tumor progression, and tumor resistance to radiation and chemotherapy. The hypoxia-inducible transcription factor (HIF), a heterodimer comprising one of two HIF- α subunits (HIF-1 α or HIF-2 α) and HIF-1 β , is the master regulator of the hypoxia response by tumors, regulating a large number of genes required for the adaptation to hypoxia. Tumor HIF-1 α is a marker of aggressive disease and poor patient prognosis in cancer patients. Consequently, HIF-1 α has been highly ranked on the list of targets for cancer therapy due to the important role it plays in regulating tumor survival and growth under hypoxic stress.

KC7F2 joins the ranks of an increasing number of reported HIF-1 α inhibitors whose diverse mechanisms includes the inhibition of either topoisomerase I, the Hsp90 molecular chaperone, microtubules, histone deacetylases (HDACs), signaling kinases or growth factor receptors (Figure 1). That a number of these proteins are also deregulated in cancer further validates HIF-1 α as a promising anti-cancer target. Additionally, the fact that the modulation of a number of unrelated molecular targets ultimately result in HIF-1 α inhibition through various mechanisms including HIF-1 α synthesis, degradation or transactivation, underscores the significance of HIF-1 α as a critical signaling hub, regulating cellular responses to a wide variety of stimuli. It is noteworthy that a large number of HIF-1 α inhibitors appear act at the level of translation. This highlights the significance of translation as a major pathway maintaining

²To whom requests for reprints should be addressed at: Garth Powis D.Phil., MD Anderson Cancer Center, 1400 Holcombe Blvd., FC6.3044, Unit 422, Houston, Texas 77030, Phone: 713-745-3366, Fax: 713-745-1710, gpowis@mdanderson.org.

¹Co-primary authors

HIF-1 α levels during hypoxia at a time when global protein translation is attenuated. However, the precise mechanism allowing preferential HIF-1 α translation during hypoxia remains unclear.

First generation drugs have shown that HIF-1 α inhibition may provide an effective antitumor strategy. The main antitumor effect of HIF-1 α inhibition appears to be through an anti-angiogenic effect mediated by the downregulation of HIF-1 α downstream targets such as the vascular endothelial growth factor (VEGF). As a result, the antitumor effects of HIF-1 α inhibitors are mostly manifested *in vivo* where angiogenesis is critical for continued tumor growth³. Narita *et.al.* show that KC7F2 is cytotoxic to cancer cells in normoxia when cells do not normally express HIF-1 α , and that KC7F2 cytotoxicity is potentiated by hypoxia. This suggests that although HIF-1 α inhibition during hypoxia may contribute to KC7F2 cytotoxicity, the cytotoxicity under normoxia likely occurs through a separate mechanism. Further characterization of KC7F2 will show whether its HIF-1 α independent toxicity could be a potential source of unwanted side-effects. It should be noted that topotecan, a topoisomerase I inhibitor that inhibits HIF-1 α translation, causes cytotoxicity by a mechanism dependent upon DNA replication-mediated DNA damage yet decreases HIF-1 α protein levels independently of DNA damage, suggesting a mechanism of HIF-1 α inhibition distinct from the one responsible for the cytotoxic effects⁴. Indeed, many HIF-1 α inhibitors have been shown to have multiple targets which may be important for their antitumor or anti-HIF-1 α activity. Additionally, many of the HIF-1 α inhibitors currently in clinical trials have some other mechanisms of action that could also rationally account for their activity such as the inhibition of targets critical for functions including cell signaling, DNA replication and cell division. For these agents it may be difficult to determine the extent HIF-1 α inhibition plays in antitumor activity. Nevertheless, some HIF-1 α inhibitors achieve their potency by inhibiting HIF-1 α at multiple levels. The guanylyl cyclase activator YC-1 inhibits HIF-1 α by promoting HIF-1 α degradation, inhibiting HIF-1 α synthesis and disrupting its transcriptional activity by interfering with the HIF-1 α /p300 interaction⁵. PX-478 a HIF-1 α inhibitor currently in Phase I clinical trial inhibits HIF-1 α by decreasing HIF-1 α translation and to a lesser extent transcription and de-ubiquitination of HIF-1 α ⁶. It is interesting to observe that KC7F2 is a disulfide compound similar to PX-12, a dual thioredoxin and HIF-1 α inhibitor that is also in early clinical trials⁷.

Narita *et.al.*, propose that KC7F2 inhibits HIF-1 α translation by reducing the phosphorylation of the translational repressor eIF4E binding protein (4E-BP1), and the ribosomal kinase, S6K and thus preventing initiation of translation. As HIF-1 α is one of the few proteins whose translation is maintained during hypoxia, and this could be a mechanism by which KC7F2 achieves selective inhibition of HIF-1 α . However, it should be noted that both 4E-BP1 and S6K, downstream targets of the mTOR pathway, are generally believed to be already inhibited in hypoxia as a means to reduce global protein translation⁸. It will be interesting to see whether KC7F2 inhibits phosphorylation of these proteins in normoxia or whether the effect of KC7F2 on the translational machinery is hypoxia-dependent.

Ultimately, although many agents have been shown to inhibit HIF-1 α in cells, only few have been shown to inhibit HIF-1 α *in vivo* and to have significant antitumor activity. The higher selectivity of KC7F2 for cancer versus normal cells and its increased cytotoxicity towards these cells in hypoxia versus normoxia is promising. Further work is needed show whether KC7F2 will live up to its potential *in vivo*.

Acknowledgements

Supported by NIH grants CA0179094, CA095060, CA0179094 and CA109552.

References

1. Narita T, Yin S, Gelin C, et al. Identification of a novel small molecule HIF-1 α translation inhibitor. *Clin Cancer Res* 2009;15
2. 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;65:605–612. [PubMed: 15695405]
3. Liao D, Johnson RS. Hypoxia: a key regulator of angiogenesis in cancer. *Cancer Metastasis Rev* 2007;26:281–290. [PubMed: 17603752]
4. Rapisarda A, Zalek J, Hollingshead M, et al. Schedule-dependent inhibition of hypoxia-inducible factor-1 α protein accumulation, angiogenesis, and tumor growth by topotecan in U251-HRE glioblastoma xenografts. *Cancer Res* 2004;64:6845–6848. [PubMed: 15466170]
5. Li SH, Shin DH, Chun YS, Lee MK, Kim MS, Park JW. A novel mode of action of YC-1 in HIF inhibition: stimulation of FIH-dependent p300 dissociation from HIF-1{ α }. *Mol Cancer Ther* 2008;7:3729–3738. [PubMed: 19074848]
6. Koh MY, Spivak-Kroizman T, Venturini S, et al. Molecular mechanisms for the activity of PX-478, an antitumor inhibitor of the hypoxia-inducible factor-1 α . *Mol Cancer Ther* 2008;7:90–100. [PubMed: 18202012]
7. Welsh SJ, Williams RR, Birmingham A, Newman DJ, Kirkpatrick DL, Powis G. The thioredoxin redox inhibitors 1-methylpropyl 2-imidazolyl disulfide and pleurotin inhibit hypoxia-induced factor 1 α and vascular endothelial growth factor formation. *Mol Cancer Ther* 2003;2:235–243. [PubMed: 12657718]
8. Barnhart BC, Lam JC, Young RM, Houghton PJ, Keith B, Simon MC. Effects of 4E-BP1 expression on hypoxic cell cycle inhibition and tumor cell proliferation and survival. *Cancer Biol Ther* 2008;7:1441–1449. [PubMed: 18708753]

Figure 1. Pathways of HIF-1α synthesis, degradation and regulation of HIF-1 activity

The HIF-1 transcription factor is heterodimer of HIF-α and HIF-1β. Under normoxic conditions HIF-α undergoes rapid pVHL-dependent proline hydroxylation followed by ubiquitination and proteasomal degradation. When HIF-α levels increase under hypoxia it enters the nucleus to combine with HIF-1β, binding to a conserved DNA sequence, the hypoxia responsive element (HRE), to transactivate a variety of hypoxia-responsive genes. Co-activators such as p300/CREB binding protein (CBP) regulate HIF-1 activity. Reported inhibitors of HIF-1 and their putative mechanism of inhibition, where known, are shown in the boxes.