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Hitting HIF1 in cancer

By Tim Fulmer, Senior Writer

Hypoxic regions in solid tumors can help trigger angiogenesis and invasion but are hard to attack with radiotherapy and chemotherapeutics because the oxygen-deprived areas are distant from any blood supply. Researchers at **The Johns Hopkins University School of Medicine** have now identified a compound, acriflavine, that directly inhibits a central player of the hypoxic response—hypoxia-inducible factor 1.[1]

Hypoxia-inducible factor 1 (HIF1) is a heterodimer consisting of the two proteins HIF1A (HIF1α) and HIF1B (HIF1β; *see* **Figure 1, "Targeting HIF1 in cancer"**). When oxygen is abundant, HIF1A is rapidly degraded by a family of three hypoxia-inducible factor prolyl hydroxylase (EGLN; HIF-PH; PHD) enzymes: PHD1, PHD2 and PHD3 (*see* **Figure 1.I[a]**). The enzymes increase HIF1A's affinity for a tumor-suppressor protein called von Hippel-Lindau (VHL) and target HIF1A to the proteasome for degradation (*see* **Figure 1.I[b]**).[2,3]

During oxygen deprivation, or hypoxia, HIF1A avoids proteasome-mediated degradation and instead accumulates in the nucleus, where it forms a dimer with HIF1B (*see* **Figure 1.II[a]**). In the presence of other transcriptional coactivators, the dimer binds specific regions of the genome to upregulate expression of hypoxia-responsive, proangiogenic genes (*see* **Figure 1.II[b]**).

In cancer, hypoxia occurs in poorly vascularized regions of solid tumors, and hypoxia-responsive genes drive prosurvival processes such as angiogenesis and tumor invasion.

Gregg Semenza and colleagues at Johns Hopkins have been using cell-based screens to help identify molecules that block HIF1 signaling in cancer. Their latest work builds on the results of a previous screen that identified 336 compounds that at 10μM blocked HIF1A-dependent gene transcription by >50% under hypoxic conditions.[4]

In a new paper published in the *Proceedings of the National Academy of Sciences*, the researchers took the top 200 hits from that screen and ran a secondary screen designed to identify compounds that reduced HIF1A-dependent gene transcription by specifically blocking HIF1A and HIF1B dimerization.

The most potent inhibitor identified by the new screen was acriflavine, a small molecule that has been known for nearly a century. The compound had an IC_{50} of about 1μM.

Previous HIF1-targeting strategies for cancer have sought to reduce HIF1A mRNA or protein levels but have never tried to block formation of the HIF1A and HIF1B dimer complex that is required for transcription of hypoxia-responsive genes.

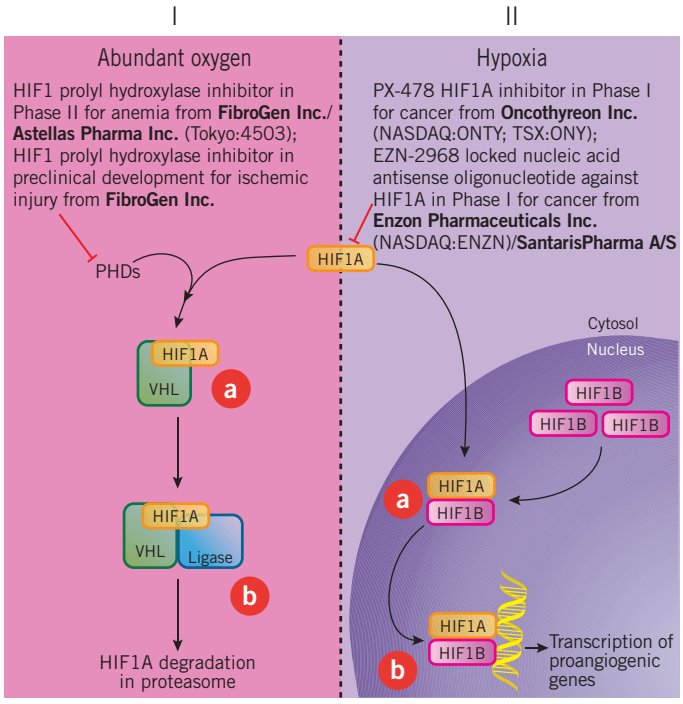

Figure 1. Targeting HIF1 in cancer.

A molecule that directly prevents dimerization should block HIF1 signaling regardless of cellular levels of the protein and thus could have efficacy in any cancer associated with hypoxia.

In cultured human embryonic kidney cells, acriflavine decreased the interaction between endogenous HIFA and HIFB under hypoxic conditions. Additional *in vitro* studies provided insight into the compound's mechanism of inhibition. Acriflavine bound to a specific subdomain of HIF1A, which prevented dimerization, blocked DNA binding and ultimately inhibited transcriptional activation of hypoxia-responsive genes.

Finally, in mouse xenograft models of prostate and liver cancer, intraperitoneal administration of acriflavine significantly reduced tumor growth compared with administration of vehicle ($p<0.01$). The compound also reduced expression of multiple proangiogenic genes within the tumor and blocked tumor vascularization compared with vehicle.

Acriflavine and its many analogs (the acridines) were first tested in humans as antimicrobials in 1917 and found wide use during World War II. However, the emergence of penicillin relegated the compounds to relative obscurity in the infectious disease field.[5] Acriflavine preparations are now marketed on the Internet to treat fungal and bacterial infections in aquarium fish.

Thus, Semenza, corresponding author and principal investigator on the paper, told *SciBX* that "availability of pharmaceutical-grade acriflavine may be an issue" to solve before moving the compound into the clinic.

The findings in the *PNAS* paper are patented and available for licensing, according to Semenza, who is director of the Institute for Cell Engineering's Vascular Program and professor of pediatrics, medicine and oncology at The Johns Hopkins University School of Medicine.

Fulmer, T. *SciBX* 2(39); doi:10.1038/scibx.2009.1461
Published online Oct. 8, 2009

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