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HIF-PROLYL hydroxylase inhibitors:

From basic science to clinical trials

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1. THE BIOLOGY OF OXYGEN SENSORS

How cells monitor local oxygen concentration is an age-old question in molecular biology. Tissues employ complex strategies to cope with oxygen deprivation, including induction of angiogenesis and alterations in energy metabolism. Extensive work performed during the past decade has demonstrated the role of a transcription factor termed HIF (hypoxia-inducible factor) as master regulator of cellular adaptation to low oxygen (1).

HIF is a member of the basic helix-loop-helix -PAS family of transcription factors and is composed of an *alpha* subunit and a constitutively expressed *beta* subunit (also known as the aryl-hydrocarbon receptor nuclear translocator: ARNT). The key to oxygen-dependent regulation is the alpha subunit, which is rapidly degraded under normoxic conditions via the proteasomal pathway and becomes stable under hypoxic conditions. Upon stabilization, HIF alpha binds ARNT and the resulting dimer targets specific DNA sequences termed hypoxia response elements (HRE), followed by the initiation of a complex transcription program, including genes involved in angiogenesis (VEGF), oxygen transport (erythropoietin), pH regulation (carbonic anhydrases IX and XII), energy metabolism (GLUT1 glucose transporter and glycolytic enzymes), nitric oxide generation (type II NOS) and cell motility (hepatocyte growth factor/scatter factor and its receptor c-met). The concerted action of these genes is thought to be critical for cell survival in low or absent oxygen in a variety of tissue types.

However, the direct oxygen sensor(s) of the HIF pathway remained elusive until 2001-2002 when two independent groups including ours (2,3,4,5) identified a novel class of oxygen-binding enzymes belonging to the class of evolutionary-conserved EGLN dioxygenases (named after the EGL9 member in Drosophila).

In normoxia and in the presence of three cofactors (ascorbic acid, iron, 2-oxoglutarate) these enzymes catalyze the addition a hydroxyl radical to specific prolines in HIF. Both biochemical and crystallography studies which have conclusively shown that this covalent modification is an absolute requirement for HIF recognition by a multiprotein complex that tags it for rapid proteasomal degradation. Conversely, EGLNs are inactivated by oxygen

deprivation, rendering HIFs unrecognizable by the destruction machinery and consequently fully active (Figure 1).

EGLNs are weakly homologous to the "classic" prolyl hydroxylases (collagen-PH), enzymes that have been known for more than 3 decades to modify prolines in collagen, being essential for its stability and tensile strength. The rather loose homology, as well as the highly unstable nature of the substrate (HIF) is highly likely to have contributed to their relatively late discovery.

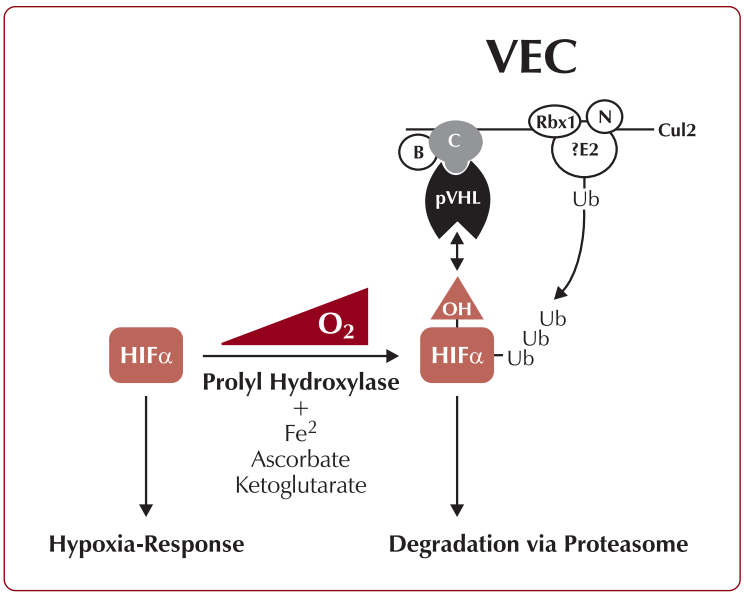

FIGURE 1. Regulation of HIF destruction by enzymatic prolyl hydroxylation. In normoxic conditions a single hydroxyl residue added on a specific proline leads to HIF recognition by a complex that contain the following proteins: the von Hippel-Lindau tumor suppressor (pVHL), Elongins B and C (labeled with B and C in the figure), Rbx1 and Cul2. This molecular interaction recruits an ubiquitin ligase (yet to be identified) that attaches a polyubiquitin chain to HIF, targeting it for degradation by the proteasomes.

In addition to the EGLN-HIF system, additional oxygen sensing mechanisms have been described, likely involved in acute responses to decreased oxygen tension. For example, acute hypoxia alters the activity of select potassium channels such as Kv3.1b, TASK1 and TASK3 could be particularly important in the airway cells, the carotid body and other arterial chemoreceptors (6). Additionally, an isoform of the neutrophil NADPH oxidase and the mitochondrial cytochrome c oxidase aa_3 are potential oxygen sensors, however their biochemical role remains unclear. ❑

2. PRELIMINARY PROMISES FOR CLINICAL APPLICATIONS

2.1. Prolyl –hydroxylase inhibitors

A variety of human disorders are characterized by, or associated with, tissue hypoxia and manipulation of the EGLN-HIF pathway has been predicted to significantly alter their clinical course. Biotechnology companies such as Fibrogen, Inc. (South San Francisco, California), are currently developing a variety of small molecule inhibitors of EGLN enzymes. A number of lead compounds have already been shown to activate HIF (and its target genes mentioned above) resulting in increased cell survival following a hypoxic injury.

Mechanistically, these blockers are designed to compete with the binding of natural EGLN cofactors (iron, ascorbate or 2-oxoglutarate). One such small molecule developed by Fibrogen, FG-4539, has been shown to be neuroprotective in preclinical models of ischemic stroke and could lead to a novel class of drugs used in cerebrovascular or cardiovascular emergencies (7).

Another inhibitor, FG-2216, provided the first demonstration of efficient erythropoietic response in humans (via induction of erythropoietin, a direct HIF target) and is now in Phase I clinical studies evaluating its efficacy in anemia of chronic kidney disease or secondary to cancer chemotherapy. Phase II trials are expected to start in the US early next year (8). ❑

2.2. HIF inhibitors

HIF overexpression in cancer is extremely frequent and seems to be required for tumor cell adaptation to the hypoxic microenvironment (reviewed in 9). Capitalizing on this notion, a several industry-based and academic groups are developing strategies for HIF inactivation. Among the most advanced is ProIX Pharmaceuticals, which developed PX-478, a small molecule inhibitor of HIF activity exhibiting potent antitumorigenic effects in preclinical studies.

The growing partnership between academia and industry in this research area is envisaged to further refine the chemical biology of the HIF pathway, with the hope of FDA-approved clinical applications in the not so distant future. ❑

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