CLINICAL IMPLICATIONS OF BASIC RESEARCH

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The Hypoxia Response Pathways — Hats Off!

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The 2016 Albert Lasker Basic Medical Research Award, announced September 13, appropriately recognizes the fundamental contributions of three leaders in the sizable field of hypoxia research for elucidating the molecular basis of oxygen-regulated gene expression. Each of the recipients — Gregg L. Semenza, William G. Kaelin, Jr., and Peter J. Ratcliffe — discovered a pathway involving a heterodimer termed hypoxia-inducible factor (HIF), which is essential and central to eukaryotic oxygen sensation.

Changes in oxygen availability represent a critical physiological stimulus for all multicellular organisms that need to match oxygen supply with the demands of respiring tissues. Beyond contributing to the maintenance of intracellular bioenergetics through the production of mitochondrial ATP, oxygen serves as a universal electron acceptor in numerous biochemical pathways. Not surprisingly, therefore, responses to hypoxia are nearly instantaneous and highly conserved throughout evolution. Hyperoxia, as well, must be protected against, for it can cause oxidative damage to lipids, proteins, and other biomolecules. Acute responses typically involve changes in the activity of existing molecules, such as ion channels, whereas more long-term adaptations include robust changes in global gene-expression programs.

In the 1990s, Semenza, a geneticist at Johns Hopkins University, and Ratcliffe, a nephrologist at Oxford University, were simultaneously investigating the transcriptional control of erythropoietin levels, which vary in response to oxygen concentrations. In 1995, Semenza and colleagues reported the biochemical purification of the two subunits of the heterodimeric "HIF-1" transcription factor: the constitutively expressed HIF-1β (which, at the time, was known as ARNT) and oxygen-regulated HIF-1α (Fig. 1).[1] Sequence information revealed that each subunit is also a member of the evolutionarily conserved superfamily of environmental sensors responding to diverse stimuli, such as light, xenobiotics, redox state, and oxygen. Research teams led by both Semenza and Ratcliffe determined that HIF is active in virtually all mammalian cells and is even conserved in primitive species that lack specialized oxygen-delivery organs. More recent efforts have shown that HIF modulates the expression of approximately 1000 genes that are broadly involved in hypoxic adaptations, including those regulating metabolism, blood-vessel growth, cell division, and inflammation.

In seemingly unrelated studies, Kaelin, a medical oncologist at the Dana–Farber Cancer Institute, investigated how pathogenic variants in the tumor-suppressor gene *VHL*, encoding the von Hippel–Lindau protein (pVHL), result in a hereditary cancer syndrome causing highly vascular tumors of the eye, central nervous system, and kidney. Working with collaborators, such as Nikola Pavletich of Memorial Sloan Kettering Cancer Center, he determined that cells lacking functional pVHL transcribe hypoxia-induced genes (e.g., *VEGF*) at high levels, irrespective of oxygen concentrations.[2] Furthermore, x-ray crystallography studies showed that a pVHL-containing multiprotein complex resembles machinery dedicated to protein turnover (through "ubiquitination") in yeast. In 1999, the Ratcliffe group discovered that pVHL-deficient renal-cancer cells fail to degrade HIF-1α and also HIF-2α, a closely related α subunit.[3] (These similar but distinct proteins are collectively described as HIF-α.) Additional studies by multiple laboratories showed that HIF-α turnover under "normal" oxygen conditions requires ubiquitin ligase activity mediated by a protein complex that includes pVHL; however, the "marks" on HIF-α proteins allowing pVHL recognition and their subsequent degradation remained mysterious.

Two landmark studies, described by Ratcliffe

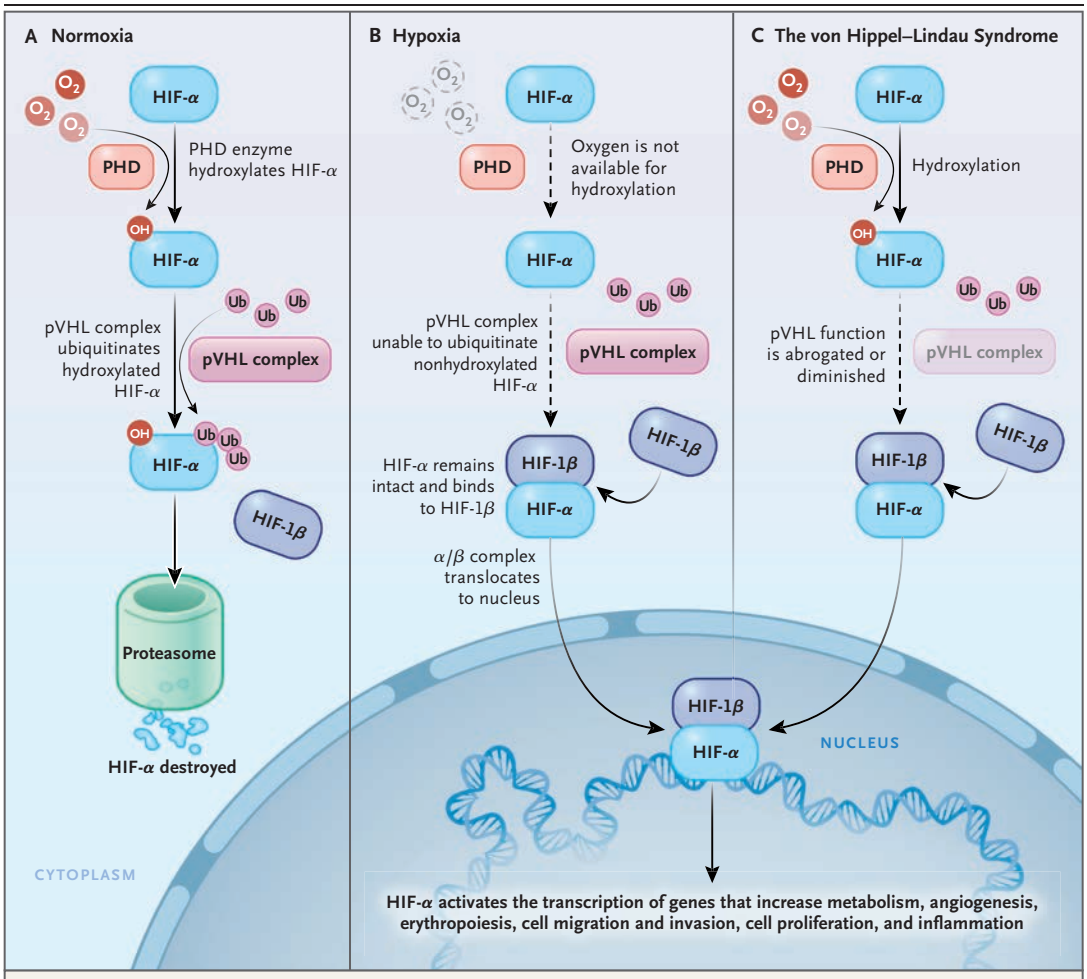

Figure 1. Making Sense of Oxygen Sensing.

The molecular machinery essential for cellular oxygen sensing is engaged when oxygen is abundant (Panel A): hypoxia-inducible factor α (HIF-α) subunits (there are two types of α subunit: HIF-1α and HIF-2α) are hydroxylated on highly conserved proline residues by a family of HIF-specific prolyl hydroxylase domain (PHD) enzymes, allowing their recognition by von Hippel–Lindau protein (pVHL)–containing ubiquitin ligase complexes. Hydroxylated HIF-α is subsequently degraded by the proteasome. As oxygen levels decline (Panel B), HIF-α prolyl hydroxylation is diminished, allowing reversible HIF-α stabilization, dimerization with HIF-1β (also known as ARNT), and target-gene stimulation. HIF-regulated genes include 500 to 1000 validated downstream effectors, depending on cellular context, that mediate a diverse array of hypoxic adaptations critical for embryonic development, normal physiology, and multiple diseases and processes, such as cancer, inflammation, and tissue ischemia. Stabilization of HIF-α and the activation of target genes also occur in the cells of persons with the von Hippel–Lindau syndrome (Panel C), which is caused by loss-of-function variants in *VHL*.

and Kaelin in 2001, showed that interactions between pVHL and the HIF-α subunits depend on the oxygen-mediated hydroxylation of two proline residues in each of the HIF-α subunits.[4,5] Using a genetic approach in the roundworm *Caenorhabditis elegans*, the Ratcliffe team found that *vhl-1* mutants constitutively express HIF-1α, as did worms deficient in a gene encoding a dioxygenase enzyme.[6] They found that this enzyme hydroxylates a highly conserved proline residue in HIF-1α, promoting HIF-1α instability when oxygen is relatively abundant, and that prolyl hydroxylation is rapidly inhibited as oxygen levels decline (Fig. 1). The amino acid sequence of the worm dioxygenase enzyme allowed the identification of three related mammalian homologues, designated "PHDs" for prolyl hydroxylase domain–containing proteins.

Collectively, extensive work led by Semenza,

Ratcliffe, and Kaelin elucidated a highly elegant mechanism whereby declining oxygen levels result in decreased HIF-α prolyl hydroxylation owing to PHD inhibition, reversible HIF-α stabilization, and the stimulation of 500 to 1000 oxygen-regulated genes mediating diverse hypoxic adaptations at the cellular, tissue, and organismal levels. Although many other intracellular processes — such as ion-channel activity, signal transduction, and oxygen-dependent biochemical reactions — "sense" oxygen availability, the HIF–PHD–pVHL axis has a central and far-reaching role in communicating external oxygen concentrations to global nuclear gene-expression programs. The extensive transcriptional output of HIF is highly integrated with additional signaling pathways, nutrient sensing, protein synthesis, and other fundamental processes dependent on cellular ATP levels. The clinical relevance of this oxygen-sensing system is substantial: hypoxia affects numerous disorders and processes, such as cancer, cardiovascular disease, pulmonary hypertension, stroke, bacterial infections, inflammation, and wound healing.

All three of these 2016 Lasker awardees have been involved in efforts to exploit such oxygen-sensing pathways for therapeutic gain. For example, Semenza did early groundwork showing the salutary effects of HIF in preclinical ischemia models, Kaelin and Ratcliffe established that the PHDs could be inhibited with drug-like molecules, and all three have probed the role of HIF in cancer. First-generation PHD inhibitors, which stabilize HIF, are being developed for renal failure, tissue ischemia, and tissue regeneration. Conversely, HIF inhibitors are being developed for cancer (especially clear-cell renal carcinoma[7]) and specific diseases such as pulmonary arterial hypertension.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

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