Cellular oxygen sensing and the anesthesiologist: the Nobel-worthy discovery of hypoxia inducible factor and its implications in clinical practice

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On October 7 2019, the Nobel committee awarded the prize in physiology or medicine to Drs Gregg Semenza (Johns Hopkins University), Peter Ratcliffe (Oxford University), and William Kaelin Jr. (Harvard University) for their discovery of hypoxia inducible factor (HIF) and associated mechanisms of oxygen sensing and biological regulation.^{1,2} The committee noted that the prize was awarded for “establishing the basis of our understanding of how oxygen levels affect cellular metabolism and physiological function”.¹ The importance of their discovery has been long understood by many across both basic science and medical fields, and was acknowledged by the Canadian Gairdner Institute in 2010.³

In many senses, this is also a definition of one of the central roles of practicing anesthesiologists—i.e., to preserve optimal perfusion and oxygen delivery to the body's tissues.

The history of oxygen discovery and its importance to physiology

The current level of oxygen in the atmosphere has evolved over the past billion years allowing complex (metazoan) organisms including humans to develop.⁴ From the earliest days, populations have been able to adapt to life at lower oxygen levels at altitude as exemplified by the Himalayan and Andean cultures. From a Western scientific perspective, the collaborative research of Carl Wilhelm Scheele (1742–1786), who first published on the nature of oxygen; Antoine Lavoisier (1743–1794), who coined the term “oxygen” as the acidic portion of the atmosphere that

supported combustion; and Joseph Priestly (1733–1804), who most clearly showed evidence that oxygen production by plants was necessary for animal survival, we have continued to focus on the importance of oxygen to support our survival.⁵ Of interest, one of the scientific discoveries linking hypoxia at altitude with changes in hemoglobin (Hb), initiated via HIF-dependent erythropoietin (EPO) expression, was achieved by Mabel Purefoy FitzGerald (honorary Master of Arts, Oxford University). As a member of Haldane's Pike's Peak Expedition, she independently provided one of the first observations of the relationship between reduced partial pressure of oxygen exposure at increased altitude and increased Hb concentration in men and women.⁶

The discovery of HIF

The discovery of HIF and its biological significance was derived from collaborative research performed over many years. The known relationship between EPO expression and hypoxia led Gregg Semenza to ask “what is the element that promotes EPO transcription under hypoxic conditions?”⁷ Ingenious investigation at the molecular level identified a “cis” element that acted at a location on the DNA ahead of the EPO gene to turn the gene on and increase gene transcription. This line of investigation led to the discovery of HIF-1, the nuclear binding factor that promoted EPO expression in isolated cells.⁸ Working in parallel, Peter Ratcliffe explored the molecular and physiologic regulation of EPO transcription and its biological expression. He determined the location of the EPO gene transcription promoter⁹ and defined the mechanism of renal EPO expression and its secretion in response to graded degrees of anemia and hypoxia.^{10,11} Finally, approaching the problem from an additional angle, William Kaelin Jr. explored the role of von Hippel Lindau

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(VHL) protein and its impact on the expression of hypoxia inducible genes under conditions of changes in ambient oxygen.¹² His research characterized the relationship between VHL and HIF by showing that binding of the two molecules only occurred if HIF was hydroxylated at its proline residues. Such hydroxylation enabled VHL binding and the subsequent destruction of the HIF-VHL protein complex via proteolytic degradation pathways in the presence of oxygen.¹³ In the absence of oxygen, such proline-hydroxylation does not occur, thus preventing VHL binding and VHL-HIF degradation resulting in the stabilization of HIF.¹⁴ Together, the discoveries made by these three researchers (and their many collaborators) led the way to a profound new understanding of the physiologic and cellular adaptation to hypoxia and cellular capacity for oxygen sensing. By virtue of this mechanism, every cell in the mammalian body has the capacity for oxygen sensing. This capacity has provided the required mechanism to drive integrative physiologic adaptations that occur in response to hypoxemia, acute blood loss, hemorrhage, and anemia frequently observed in our acute care patients.

Discovery through collaboration

In a world of ever-increasing competition, with an over-emphasis on becoming “independent”, the research programs that evolved from the mutual discoveries of these three leaders can only be described as truly collaborative. Indeed, one member of the Nobel committee described the co-dependent interaction between these investigators as a “three-legged stool”, emphasizing that each leg was essential to achieve the discovery.² In reference to the value of this collaboration and discovery, William Kaelin Jr. eloquently summarized the impact as follows: “Occasionally you understand something that has never been understood before, and if you are really lucky you might discover a mechanism that is so beautiful that it makes your jaw drop. That is the real prize—to be able to participate in that process”.¹

How might this discovery impact our practice as anesthesiologists?

Our understanding of HIF biology, and its role in regulating oxygen homeostasis, has incorporated an expanding number of biological systems and is known to involve over 4,000 genes.¹ As our understanding continues to evolve, the potential for therapeutic intervention grows. One example of how this biology may help improve the health and well-being of our patients may be the effective treatment of acute and

chronic anemia. Anemia is a global health problem. Indeed, an estimated one third of the world’s population suffers from anemia, which is a leading cause of disability in youth and young adults.^{15,16} Despite this known baseline risk, an estimated 30% of patients undergoing elective surgical procedures have been identified as being anemic.¹⁷ Furthermore, we know that even mild to moderate anemia is associated with an increased risk for perioperative morbidity and mortality in patients undergoing elective cardiac and non-cardiac surgery.^{17,18} Finally, acute hemodilution and blood loss, which frequently occur during surgery, are also associated with a further increased incidence of severe adverse outcomes.¹⁹

Thus, anemia is an identified risk to our patients. But, what can we do to improve patient outcomes? First, understanding anemia as a risk²⁰ has supported the development of effective multidisciplinary blood management programs, such as the Ontario Network of Transfusion Coordinators (ONTraC), which have been shown to effectively treat anemia, minimize blood transfusion, and improve patient outcomes.²¹ Second, defining anemia as a risk has re-focused research on the importance of oxygen delivery to tissue, and its limitation, as a potential mechanism of injury. From human and animal studies,^{22,23} we know that the true conditions of tissue oxygen delivery cannot be successfully mimicked in the test tube, or fully defined by studies in cultured cells. Thus, accurate assessment of the impact of anemia on tissue hypoxia must be performed using integrative whole organism physiological approaches. Utilizing whole animal transgenic models derived from knowledge of the HIF discovery,²⁴ we understand that anemia leads to multi-organ tissue hypoxia and enhanced HIF expression in tissues of anemic animals.²³ Furthermore, interrupting HIF expression has been shown to increase mortality in animal models of acute anemia,²³ indicating that HIF-dependent mechanisms support survival during anemia. Thirdly, clinical research has assessed whether measuring levels of hypoxic serum biomarkers, such as EPO, in the blood can predict the risk of tissue hypoxia and organ injury (e.g., the kidney).²⁵ This approach may help to identify anemic patients at risk of acute organ injury and direct effective treatments, including treatment of pre-operative anemia and targeted blood transfusion, in patients at risk who experience acute surgical blood loss.

Understanding the biology of HIF-dependent transcription of EPO, and the role of proline-hydroxylation in the degradation of HIF has led to the development and assessment²⁴ of novel peptides that can inhibit prolyl-hydroxylase enzymes responsible for HIF degradation, thereby stabilizing HIF and increasing the expression of HIF-dependent molecules such as EPO.²⁶ This approach may directly promote erythropoiesis as a primary goal to treat anemia. Completed phase-III clinical trials have shown the efficacy of these novel oral peptide drugs with respect to

treating anemia and improving Hb levels.²⁶ This new class of drugs may both treat anemia and enhance hypoxic cellular defense mechanisms to enhance tolerance of anemia and improve patient outcomes. Nevertheless, close examination for potential adverse effects of generalized enhancement of HIF-dependent gene expression must also be maintained.

In summary, the discovery of HIF and its multiple integrated physiologic mechanisms is of great value to our understanding of human biology. It lends to a greater understanding of the importance of how our complex biological systems regulate and utilize oxygen under times of plenty and times of need. Ongoing and future studies will be required to determine whether or not, therapeutic interventions based on this knowledge will improve patient outcomes and quality of life. As anesthesiologists, we remain focused on maintaining optimal oxygen delivery to vital organs through an understanding of the integrated physiologic function of all organ systems in the acute care of patients who are frequently exposed to the risk of hypoxia and acute blood loss. As such, we are well-positioned to contribute to the ongoing application of new treatments to optimize patients' outcomes, in part, based on our understanding of the risk of tissue hypoxia and the role of HIF biology in ensuring adequate oxygen delivery to tissues.

Conflicts of interest None.

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