

Nobel Prize in Physiology or Medicine 2019*

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The Nobel Prize in Physiology or Medicine, 2019 was awarded jointly to William G. Kaelin Jr. of Dana-Farber Cancer Institute, Sir Peter J. Ratcliffe of Francis Crick Institute and University of Oxford and Gregg L. Semenza of Johns Hopkins University for their discovery of how cells sense and respond to varying oxygen levels.

Cells derive energy by burning ‘food’ with oxygen. For vertebrates, including humans, respiration brings oxygen into the blood, which then is carried to every cell. It is long known that the respiratory center in the brain controls breathing – lower oxygen content in blood alters breathing to bring more oxygen into circulation. The carotid body, present in a sinus at the base of the inner carotid artery in the neck, functions as a chemosensor for lower oxygen content in blood. Carotid body communicates with the respiratory center to regulate respiration – a discovery for which Nobel Prize for Physiology or Medicine was awarded in 1938 [1]. At the cellular level, lowered oxygen concentration (hypoxia) brings about a lot of gene expression changes as an adaptive response. Gene expression changes alter the metabolism of the cell experiencing hypoxia. However, how cells sense and respond to changes in oxygen levels was not known until the discoveries of this year’s winners. Nobel Prize in Physiology or Medicine, 2019 was awarded jointly to William G. Kaelin Jr, Sir Peter J. Ratcliffe and Gregg L. Semenza who discovered the molecular pathways that explain how cells sense oxygen levels and respond to hypoxic environments [2].

The Nobel Laureates 2019 discovered the proteins and their mechanism/s of action (*Figure 1*) involved in oxygen sensing and response pathways within the cell. The key proteins that govern



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Keywords

Hypoxia, normoxia, hypoxia inducible factor (HIF), hypoxia responsive element (HRE), angiogenesis, erythropoietin (EPO), HIF-prolyl hydroxylase, von Hippel Lindau (VHL).

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oxygen signaling include Hypoxia Inducible Factor (HIF), HIF-Prolyl Hydroxylase (HIF-PH) and von Hippel Lindau protein (pVHL).

Discovery of Hypoxia Inducible Factor (HIF)

Gregg Semenza identified a regulatory sequence (HRE – Hypoxia Responsive Element) adjacent to erythropoietin (*EPO*) gene. Erythropoietin is a hormone that stimulates red blood cell production (erythropoiesis). Semenza further discovered that the protein complex that binds at HRE played a critical role in increasing *EPO* production at low oxygen levels [3]. This protein complex was highly inducible under hypoxia and Semenza called the protein complex ‘Hypoxia Inducible Factor’ (HIF) [4]. Peter Ratcliffe and Gregg Semenza studied *EPO* gene expression under hypoxia and found that response to hypoxia is active in different cell types [5]. The HIF is a transcription factor and consists of two subunits – HIF1 alpha and HIF1 beta (also known as Aryl Hydrocarbon Receptor Nuclear Translocator, ARNT) [6]. HIF1 beta is constitutively expressed; its expression is not dependent on oxygen levels and is localized in the nucleus. The stability of HIF1 alpha alone gets altered in response to oxygen levels. Under hypoxia, HIF1 alpha subunit gets stabilized, moves to the nucleus, and forms heterodimer with HIF1 beta. The HIF1 alpha/beta dimer binds to the HRE on DNA to activate the expression of several genes responsible for maintaining the cellular functions under hypoxia [4]. Under normal oxygen levels (normoxia), HIF1 alpha subunit is rapidly degraded. HIFs are now considered as the master regulators of cellular response to hypoxia, and they control several processes including fetal development, erythropoiesis, angiogenesis and tumor cellular metabolism [7].

Under normal oxygen levels (normoxia), ubiquitin is added to HIF1 alpha to mark it for degradation by the proteasome and HIF1 alpha subunit is rapidly degraded.

Link Between HIF1 alpha and pVHL

The pursuit to find how cells sense hypoxia and stabilize HIF1 alpha was continuing. Around the same time, it was identified that under normal oxygen conditions, ubiquitin is added to HIF1 alpha to mark it for degradation by the proteasome [8]. Which



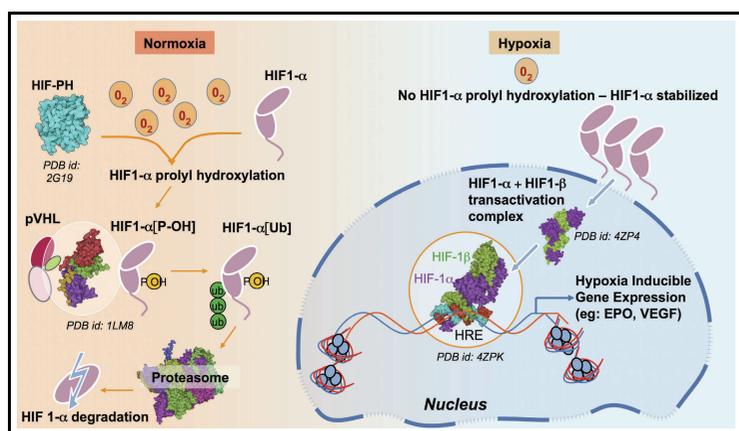


Figure 1. Nobel Prize 2019 for Physiology or Medicine is awarded for explaining cellular oxygen sensing and response. At normoxia (normal physiological oxygen concentration), hypoxia inducible factor 1 alpha (HIF1- α) is hydroxylated by HIF-prolyl hydroxylase (HIF-PH). Prolyl hydroxylated HIF1- α is ubiquitinated by von Hippel Lindau protein (pVHL) complex, then degraded via proteasome. Under low oxygen levels (hypoxia), HIF1- α gets stabilized, moves to the nucleus, and forms complex with HIF1- β . HIF1- α - β transactivation complex binds hypoxia responsive element (HRE) in hypoxia inducible genes such as *EPO* and *VEGF*, which are responsible for hypoxic adaptation.

protein/s play a role in the ubiquitination of HIF1 alpha under normal oxygen conditions came from the work of William Kaelin and Peter Ratcliffe. While working on VHL (von Hippel Lindau) disease, Kaelin's group observed that the presence of mutations in the *VHL* gene led to increased HIF activity. Mutations in *VHL* gene lead to hereditary von Hippel Lindau disease and the patients with *VHL* gene mutations are at an increased risk for certain cancers, particularly kidney cancer. The pVHL (VHL protein) is a tumor suppressor and exists as part of a protein complex that possesses E3 ubiquitin ligase activity. They showed that pVHL binds the hydroxylated HIF1 alpha under normoxia, which facilitates its ubiquitination and degradation via proteasome. (The discovery of ubiquitin-mediated proteasomal degradation won the Nobel Prize in Chemistry, 2004.) [9]. Peter Ratcliffe's group further showed that cells deficient in pVHL accumulated high levels of HIF1 alpha subunit [10].

HIF-Prolyl Hydroxylase (HIF-PH): An Oxygen-sensing Enzyme

The link between oxygen levels and HIF1 alpha stability came in 2001. Ratcliffe and Kaelin, along with others, simultaneously identified that the HIF1 alpha subunit undergoes hydroxylation on specific proline residues by the action of HIF-prolyl hydroxy-

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lase (HIF-PH) under normoxia. The resulting hydroxylated HIF1 alpha is marked for proteolysis via the cellular proteasome machinery with the help of pVHL-mediated ubiquitination. Thus, HIF-PH acts as an oxygen sensor and has a critical role in regulating the stability of HIF1 alpha and HIF-mediated target gene expression [11, 12]. The HIF-prolyl hydroxylases become inactive under hypoxia and can no longer hydroxylate HIF1 alpha. Thus, HIF1 alpha gets stabilized, translocates to the nucleus, and forms a heterodimer with HIF1 beta. The HIF1 alpha and beta complex binds to hypoxia responsive elements and regulates the expression of genes under low oxygen conditions. These discoveries by this year's Nobel Laureates explained how several genes involved in angiogenesis (Ex: Vascular Endothelial Growth Factor – VEGF), erythropoiesis (Ex: EPO) and glucose metabolism are regulated under hypoxia.

Hypoxia in Health and Disease

Response to low oxygen is a fundamental phenomenon in both normal physiological functions such as high-altitude adaptation and embryonic development as well as several diseases.

Response to low oxygen is a fundamental phenomenon in both normal physiological functions such as high-altitude adaptation and embryonic development as well as several diseases. Hypoxia has been observed in numerous diseases including cancer, anemia, retinopathy of prematurity, stroke, etc. Rapidly proliferating tumors especially solid tumors, exhibit hypoxia and elevated expression of HIF1 alpha. The genes regulated by HIF1 alpha facilitate tumor angiogenesis, altered tumor cell metabolism and metastasis. Tumors with elevated HIF1 alpha levels often show resistance to chemo and radiotherapy, and as a result, they often result in poor patient outcomes.

Implications

This year's Nobel Prize-winning work has wide-ranging implications. Low levels of oxygen or hypoxia could be beneficial or detrimental based on the physiological or disease state [13]. Stabilizing HIF1 alpha in anemia or ischemia is beneficial to the patient as high HIF1 alpha levels promote angiogenesis and hence



Box 1. Glossary

Hypoxia: The cell/tissue environment where the oxygen levels are insufficient for performing normal physiological functions.

Normoxia: The state in which cells experience normal levels of oxygen required for healthy functioning.

Transcription Factor: A protein that binds to a specific DNA sequence and regulates the synthesis of RNA from DNA. Transcription factors ensure that genes are expressed in the right cellular context.

Hypoxia inducible factor (HIF): A transcription factor that gets stabilized under hypoxia. HIF consists of two protein subunits, HIF1 alpha and beta and binds to hypoxia responsive element (HRE) to regulate the transcription of several hundred genes in response to low tissue-oxygen levels.

HIF-Prolyl Hydroxylase: An oxygen-sensing enzyme that adds a hydroxyl (-OH) group to specific proline residues of HIF1 alpha protein under normoxia for marking it for proteolysis.

von Hippel Lindau Protein (pVHL): A tumor suppressor protein. Mutations in the *VHL* gene cause certain types of cancer. pVHL is part of a protein complex that facilitates the ubiquitination of proteins and thus mediate their proteolysis.

Angiogenesis: The process of formation of new blood vessels. Angiogenesis plays an important role in cancer metastasis.

Vascular Endothelial Factor (VEGF): An angiogenic-signaling factor that promotes the formation of new blood vessels.

Erythropoiesis: The process of production of red blood cells (RBCs) or erythrocytes.

Erythropoietin (EPO): A hormone that stimulates RBC production. EPO is produced in response to low oxygen levels, mostly in the kidneys and transported to the bone marrow where it stimulates the production of RBC.

adaptation under low oxygen conditions. Accordingly, HIF-PH inhibitors have been developed and tested in patients to treat anemia. Roxadustat is one such HIF-PH inhibitor [14] currently used to treat anemia related to chronic kidney disease in China and under clinical trials in other parts of the world. On the contrary, efforts are being made in cancer to inhibit the functions of HIF and its targets. Of particular interest are HIF targets, VEGF and metabolic enzymes. US Food and Drug Administration (USFDA) approved several VEGF inhibitors for the treatment of cancers. Currently, research is ongoing to find specific inhibitors for several of HIF target genes.



Suggested Reading

- [1] <https://www.nobelprize.org/prizes/medicine/1938/summary/>
- [2] <https://www.nobelprize.org/prizes/medicine/2019/summary/>
- [3] G L Semenza, M K Nejfelt, S M Chi, and S E Antonarakis, Hypoxia-inducible nuclear factors bind to an enhancer element located 3' to the human erythropoietin gene, *Proc Natl Acad Sci U S A.*, Vol.88, pp.5680–5684, 1991b.
- [4] G L Semenza and G L Wang, A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation, *Mol. Cell Biol.*, Vol.12, pp.5447–5454, 1992.
- [5] G L Wang and G L Semenza, General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia, *Proc Natl Acad Sci USA.*, Vol.90, pp.4304–4308, 1993.
- [6] G L Wang and G L Semenza, Purification and characterization of hypoxia-inducible factor 1, *J Biol Chem.*, Vol.270, pp.1230–1237, 1995.
- [7] J C Chappell, L B Payne and W K Rathmell, Hypoxia, angiogenesis, and metabolism in the hereditary kidney cancers, *J Clin Invest*, Vol.129 pp.442–451, 2019.
- [8] S Salceda and J Caro, Hypoxia-inducible factor 1alpha (HIF-1alpha) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes, *J Biol Chem.*, Vol.272, pp.22642–22647, 1997.
- [9] <https://www.nobelprize.org/prizes/chemistry/2004/summary/>
- [10] P H Maxwell, M S Wiesener, G W Chang, S C Clifford, E C Vaux, M E Cockman, C C Wykoff, C W Pugh, E R Maher and P J Ratcliffe, The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis, *Nature*, Vol.399, pp.271–275, 1999.
- [11] P Jaakkola, D R Mole, Y M Tian, M I Wilson, J Gielbert, S J Gaskell, A von Kriegsheim, H F Hebestreit, M Mukherji, C J Schofield, P H Maxwell, C W Pugh and P J Ratcliffe, Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation, *Science*, Vol.292, pp.468–472, 2001.
- [12] M Ivan, K Kondo, H Yang, W Kim, J Valiando, M Ohh, A Salic, J M Asara, W S Lane and W G Kaelin Jr, HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing, *Science*, Vol.292 pp.464–468, 2001.
- [13] G L Semenza, Oxygen sensing, hypoxia-inducible factors, and disease pathophysiology, *Annu Rev Pathol.*, Vol.9, pp.47–71, 2014.
- [14] N Chen, C Hao, B C Liu BC, H Lin, C Wang, C Xing, X Liang, G Jiang, Z Liu, X Li, L Zuo, L Luo, J Wang, M H Zhao, Z Liu, G Y Cai, L Hao, R Leong, C Wang, C Liu, T Neff, L Szczech and K P Yu, Roxadustat treatment for anemia in patients undergoing long-term dialysis, *N Engl J Med*, Vol.381, pp.1011–1022, 2019.

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