COMMENTARY

Oxygen sensing and adaptability won the 2019 Nobel Prize in Physiology or medicine

Qing Zhang a,*, Qin Yan b,**, Haifeng Yang c,***, Wenyi Wei d,****

a Department of Pathology, UT Southwestern Medical Center, Dallas, TX, USA
b Department of Pathology, Yale Cancer Center, Yale Stem Cell Center, Yale School of Medicine, New Haven, CT, USA
c Department of Pathology, Anatomy & Cell Biology, Thomas Jefferson University, Philadelphia, USA
d Department of Pathology, Harvard Medical School, Boston, MA, USA

Received 14 October 2019; accepted 14 October 2019
Available online 19 October 2019

Abstract The 2019 Nobel Prize in Physiology or Medicine was awarded to three physician scientists, Drs. William G. Kaelin, Jr., Peter Ratcliffe and Gregg Semenza, for their groundbreaking work revealing how cells sense and adapt to oxygen availability. Here, we summarize the history of their discoveries.

The 2019 Nobel Prize in Physiology or Medicine was awarded to three physician scientists, Drs. William G. Kaelin, Jr., Peter Ratcliffe and Gregg Semenza, for their groundbreaking work revealing how cells sense and adapt to oxygen availability. We are fortunate to have had the opportunity to work in Dr. William Kaelin’s laboratory as postdoctoral trainees (Fig. 1). Here, we provide a brief description of the history and timeline of Dr. Kaelin’s research, as well as that by Drs. Ratcliffe and Semenza.
Oxygen is vital for all living organisms. During the course of evolution, animals have developed the ability to adapt to changes in the oxygen concentration on earth. However, it was unclear how animals can sense and adapt to changes in oxygen availability until around thirty years ago. The pioneering work performed by the laboratories of Drs. William Kaelin, Jr., Peter Ratcliffe and Gregg Semenza paved the way to understand the molecular mechanism of oxygen sensing. We herein summarize the major milestones in the history of research on the oxygen sensing pathway (Fig. 2).

The journey started with the purification of erythropoietin (EPO), a glycoprotein hormone produced by the fetal liver and then by adult kidneys. The protein was purified in 1977 and the gene was cloned in 1985. At that time, it was known that EPO was produced in response to a low blood oxygen concentration. However, it was not known how EPO was regulated by low oxygen. Semenza and his colleagues found that a region located on the 3' enhancer of EPO, currently known as the Hypoxia Response Element (HRE), was responsible for nuclear factor binding and EPO expression under hypoxia. This finding was subsequently confirmed in the same year by Ratcliffe and his colleagues. In addition, Ratcliffe and colleagues found that HRE DNA binding can occur in all cell types, including those not involved in EPO
vascular endothelial growth factor (VEGF).\textsuperscript{6,7} Encoding glycolytic enzymes and the angiogenic factor, HIF, it became clear that VHL is a landmark paper published in 2001,\textsuperscript{15,16} they demonstrated the close relationship between VHL and HIF. Indeed, in two back-to-back landmark papers published in 2001,\textsuperscript{15,16} they showed that VHL could lead to VEGF stabilization and subsequent increased vascularity. Indeed, he soon discovered that VHL loss of function led to upregulation of VEGF and other HIF target genes, even under normoxia.\textsuperscript{12} The E3 ubiquitin ligase activity of the VHL complex was subsequently demonstrated.\textsuperscript{10,11} The fact that the mRNA expression of both subunits typically remains constant under either normoxia or hypoxia, the HIF-1α protein was found to be induced and accumulate under hypoxia, suggesting that some type of post-transcriptional/post-translational modification(s) contribute to the regulation of HIF-1α at the protein level. Kaelin’s group at Harvard made major contributions to deciphering this regulatory mechanism. Based on his clinical experience as an oncologist, Kaelin was aware that kidney tumors that lose expression of the von Hippel-Lindau (VHL) tumor suppressor are highly vascularized. When he and his group found that VHL forms a complex with Elongin B and C and CUL2, homologs of the yeast ubiquitin ligase proteins,\textsuperscript{10,11} he hypothesized that the absence of VHL could lead to VEGF stabilization and subsequent increased vascularity. Indeed, he soon discovered that VHL deficiency led to upregulation of VEGF and other HIF target genes, even under normoxia.\textsuperscript{12} The E3 ubiquitin ligase activity of the VHL complex was subsequently demonstrated,\textsuperscript{10,11} thus establishing a key role for VHL in the regulation of angiogenesis. The discovery of VHL prolyl and asparaginyl hydroxylation completed the elegant network of oxygen-modulated regulation of HIF-1 activity. Interestingly, FIH-1 can be enzymatically active at a lower oxygen concentration than PHDs.\textsuperscript{24} FIH-1 hydroxylates asparagine 803, which is located in the C-terminal transactivation domain of HIF-1α, thereby inhibiting HIF-1α transcriptional activity by preventing its interaction with transcriptional coactivators.\textsuperscript{23}
complex, which leads to HIF-1α ubiquitination and subsequent proteasomal degradation (Fig. 3).

In less than a decade, Kaelin, Ratcliffe and Semenza delineated the detailed molecular mechanism by which the oxygen sensing pathway works. It is important to point out that these three outstanding scientists worked independently and solved many puzzles from different perspectives: Kaelin started his line of research from his experiences in oncology and based on biochemical investigations; Ratcliffe’s studies started from his role as a nephrologist, and Semenza utilized his expertise in medical genetics. Their Nobel prize-winning work embodies the importance of cross-disciplinary approaches in tackling critical questions in physiology and human diseases.

Importantly, their work opened up the important field of research focused on mammalian oxygen sensing. At present, there are close to 70 enzyme family members that may depend on oxygen for their functions besides prolyl hydroxylases.

In summary, Drs. Kaelin, Ratcliffe and Semenza paved the road for understanding oxygen sensing and adaptability, and major efforts are currently underway to develop therapeutic strategies to treat human diseases such as anemia, coronary artery disease, inflammatory bowel diseases and cancer.

Acknowledgements

We are eternally grateful for the invaluable guidance provided by Dr. William Kaelin of the Dana-Farber Cancer Institute of Harvard Medical School during our memorable postdoctoral training. We also thank the Science Bulletin in China for granting permission for the reprint. We apologize to the numerous investigators whose important relevant publications cannot be cited due to space constraints. The research conducted in the authors’ laboratories was supported in part by grants from the American Cancer Society Research Scholar Award (to Q. Z), the National Institutes of Health (R01CA211732 to Q. Z. and R01CA237586 and P50CA121974 (to Q. Y.)), the Cancer Prevention & Research Institute of Texas (to Q. Z), and the VHLA award (to H. Y.). The funding sources were not involved in the study design, in the collection, analysis and interpretation of data, in the writing of the report, or in the decision to submit the paper for publication.

References

1. Miyake T, Kung CK, Goldwasser E. Purification of human erythropoietin. J Biol Chem. 1977;252:5558–5564.
2. Lin FK, et al. Cloning and expression of the human erythropoietin gene. Proc Natl Acad Sci U S A. 1985;82:7580–7584. https://doi.org/10.1073/pnas.82.22.7580.
3. Semenza GL, Nejfelt MK, Chi SM, Antonarakis SE. Hypoxia-inducible nuclear factors bind to an enhancer element located 3′ to the human erythropoietin gene. In: Proceedings of the National Academy of Sciences of the United States of America. vol. 88. 1991:5680–5684.

4. Pugh CW, Tan CC, Jones RW, Ratcliffe PJ. Functional analysis of an oxygen-regulated transcriptional enhancer lying 3′ to the mouse erythropoietin gene. In: Proceedings of the National Academy of Sciences of the United States of America. vol. 88. 1991:10553–10557. https://doi.org/10.1073/pnas.88.23.10553.

5. Maxwell PH, Pugh CW, Ratcliffe PJ. Inducible operation of the erythropoietin 3′ enhancer in multiple cell lines: evidence for a widespread oxygen-sensing mechanism. Proc Natl Acad Sci U S A. 1993;90:2423–2427. https://doi.org/10.1073/pnas.90.6.2423.

6. Firth JD, Ebert BL, Pugh CW, Ratcliffe PJ. Oxygen-regulated control elements in the phosphoglycerate kinase 1 and lactate dehydrogenase A genes: similarities with the erythropoietin 3′ enhancer. Proc Natl Acad Sci U S A. 1994;91:6496–6500. https://doi.org/10.1073/pnas.91.14.6496.

7. Forsythe JA, et al. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol Cell Biol. 1996;16:4604–4613. https://doi.org/10.1128/mcb.16.9.4604.

8. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci U S A. 1995;92:5510–5514. https://doi.org/10.1073/pnas.92.12.5510.

9. Wang GL, Semenza GL. Purification and characterization of hypoxia-inducible factor 1. J Biol Chem. 1995;270:1230–1237. https://doi.org/10.1074/jbc.270.3.1230.

10. Kibel A, Ililopoulos O, DeCaprio JA, Kaelin Jr WG. Binding of the von Hippel-Lindau tumor suppressor protein to Elongin B and C. Science. 1995;269:1444–1446. https://doi.org/10.1126/science.7660130.

11. Lonergan KM, et al. Regulation of hypoxia-inducible mRNAs by the von Hippel-Lindau tumor suppressor protein requires binding to complexes containing elongins B/C and Cul 2. Mol Cell Biol. 1998;18:732–741. https://doi.org/10.1128/mcb.18.2.732.

12. Ililopoulos O, Levy AP, Jiang C, Kaelin Jr WG, Goldberg MA. Negative regulation of hypoxia-inducible genes by the von Hippel-Lindau protein. Proc Natl Acad Sci U S A. 1996;93:10595–10599. https://doi.org/10.1073/pnas.93.20.10595.

13. Kamura T, et al. Rbx1, a component of the VHL tumor suppressor complex and SCF ubiquitin ligase. Science. 1999;284:657–661. https://doi.org/10.1126/science.284.5414.657.

14. Kamura T, et al. Activation of HIF1alpha ubiquitination by a reconstituted von Hippel-Lindau (VHL) tumor suppressor complex. Proc Natl Acad Sci U S A. 2000;97:10430–10435. https://doi.org/10.1073/pnas.190332597.

15. Ivan M, et al. HIFalpha targeted for HIF-mediated destruction by proline hydroxylation: implications for O2 sensing. Science. 2001;292:464–468. https://doi.org/10.1126/science.1059817.

16. Jaakkola P, et al. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. Science. 2001;292:468–472. https://doi.org/10.1126/science.1059796.

17. Brueck RK, McKnight SL. A conserved family of prolyl-4-hydroxylases that modify HIF. Science. 2001;294:1337–1340. https://doi.org/10.1126/science.1066373.

18. Epstein AC, et al. C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. Cell. 2001;107:43–54. https://doi.org/10.1016/s0092-8674(01)00507-4.

19. Ivan M, et al. Biochemical purification and pharmacological inhibition of a mammalian prolyl hydroxylase acting on hypoxia-inducible factor. In: Proceedings of the National Academy of Sciences of the United States of America. vol. 99. 2002:13459–13464. https://doi.org/10.1073/pnas.192342099.

20. Jiang BH, Zheng JZ, Leung SW, Roe R, Semenza GL. Trans-activation and inhibitory domains of hypoxia-inducible factor 1 alpha. Modulation of transcriptional activity by oxygen tension. J Biol Chem. 1997;272:19253–19260. https://doi.org/10.1074/jbc.272.31.19253.

21. Pugh CW, O’Rourke JF, NagaO, Gleadle JM, Ratcliffe PJ. Activation of hypoxia-inducible factor-1: definition of regulatory domains within the alpha subunit. J Biol Chem. 1997;272:11205–11214. https://doi.org/10.1074/jbc.272.17.11205.

22. Mahon PC, Hirota K, Semenza GL. FIH-1: a novel protein that interacts with HIF-1 alpha and VHL to mediate repression of HIF-1 transcriptional activity. Genes Dev. 2001;15:2675–2686. https://doi.org/10.1101/gad.924501.

23. Lando D, et al. FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. Genes Dev. 2002;16:1466–1471. https://doi.org/10.1101/gad.991402.

24. Flashman E, et al. Evidence for the slow reaction of hypoxia-inducible factor prolyl hydroxylase 2 with oxygen. FEBS J. 2010;277:4089–4099. https://doi.org/10.1111/j.1742-4658.2010.07804.x.

25. Losman JA, Kaelin Jr WG. What is the difference a hydroxyl makes: mutant IDH, (R)-2-hydroxyglutarate, and cancer. Genes Dev. 2013;27:836–852. https://doi.org/10.1101/gad.217406.113.

26. Klose RJ, et al. The retinoblastoma binding protein RBP2 is an H3K4 demethylase. Cell. 2007;128:889–900. https://doi.org/10.1016/j.cell.2007.02.013.

27. Chakraborty AA, et al. Histone demethylase KDM6A directly senses oxygen to control chromatin and cell fate. Science. 2019;363:1217–1222. https://doi.org/10.1126/science.aaw1026.

28. Battie M, et al. Hypoxia induces rapid changes to histone methylation and reprograms chromatin. Science. 2019;363:1222–1226. https://doi.org/10.1126/science.aau5870.

29. Zhang J, et al. VHL substrate transcription factor ZHK2 as an oncogenic driver in clear cell renal cell carcinoma. Science. 2018;361:290–295. https://doi.org/10.1126/science.aap8411.

30. Zheng X, et al. Prolyl hydroxylation by Egln2 destabilizes FOXO3a by blocking its interaction with the USP9x deubiquitinase. Genes Dev. 2014;28:1429–1444. https://doi.org/10.1101/gad.242131.114.

31. Cho H, et al. On-target efficacy of a HIF2alpha antagonist in preclinical kidney cancer models. Nature. 2016. https://doi.org/10.1038/nature19795.

32. Chen W, et al. Targeting renal cell carcinoma with a HIF-2 antagonist. Nature. 2016. https://doi.org/10.1038/nature19796.

33. Zurlo G, Zhang Q. The history of oxygen sensing: 2016 lasker medical research. Sci Bull. 2016:61:1665–1668. https://doi.org/10.1007/s11434-016-1181-0.