THE INSPIRED PHYSIOLOGIST

Peter Ratcliffe's landmark discovery of how cells sense and respond to the availability of oxygen has transformed our understanding of cancer and other diseases—and he's far from done with the discovering.

Doctors pick their specialties for all sorts of reasons. Peter Ratcliffe, for his part, suspects he might have been flattered into his.

While a house officer—or resident—at a London hospital in the late 1970s, Ratcliffe worked for a time under the supervision of a respected nephrologist. While on rounds one day, he recalls, the senior doctor complimented him on his grasp of nephrology and suggested he specialize in the field. "He was an inspiring person, and I believed him," says Ratcliffe. Other senior colleagues, however, were less sanguine. The UK National Health Service was as short on cash as ever and funding for expensive renal specialists was unlikely to ever be placed high on the list of priorities. "They said, 'Good luck,'" Ratcliffe recalls, "'you'll have to distinguish yourself'."

Ratcliffe evidently took that suggestion as well. By the early 90s—having moved to Oxford to study renal medicine—Ratcliffe was among the leaders in a trans-Atlantic race to find the molecular sensor by which animal cells respond to oxygen starvation, or hypoxia. His efforts contributed not only to the discovery of that crucial sensor but to the illumination of an entirely new mechanism of intracellular signaling as well. For these discoveries and their contributions to our understanding and potential treatment of disorders ranging from anemia to heart disease and cancer, Ratcliffe was knighted in 2014 and shared with U.S. researchers William Kaelin and Gregg Semenza the prestigious 2016 Albert Lasker Basic Medical Research Award.

Ratcliffe, meanwhile, has dug deeper into the cell's oxygen sensing systems at his lab in Ludwig Oxford. In 2018, he and his colleagues detailed in *EMBO Reports* the interactions of two controlling elements of that system—hypoxia inducible factor-

PETER RATCLIFFE

LUDWIG OXFORD

Photo by Paul Wilkinson

Ratcliffe in his Oxford lab with postdoc Norma Masson.

Photo by Paul Wilkinson

1α (HIF-1α) and HIF-2α—across the entire genome. Most notably, he and his team also put the finishing touches on a study, published in 2019 in *Science*, describing an entirely new system of oxygen sensing so fundamental to cell biology that it is shared by plants and animals.

"Like many things, that we actually did this work owed a lot to serendipity," says Ratcliffe. "But part of that serendipity was the support I received from the Ludwig Institute to do something different. This was one of those things."

Stumbling into a calling

Ratcliffe grew up in a small railway town in Lancashire named Carnforth, where his father was a lawyer and his mother a homemaker. When he was close to graduating from Lancaster Royal Grammar School, intent on someday becoming an industrial chemist, the head master—an austere, begowned sort—wandered into his chemistry lab. Calling him aside, he said, "'Ratcliffe, I think you should study medicine'," Ratcliffe recalls. "To this day, I have no idea why he said that, but he was not the sort of guy you challenged so I immediately said, 'yes, sir,' and changed my university application from chemistry to medicine."

Ratcliffe won a scholarship in 1972 to study medicine at Gonville & Caius College,

Cambridge, and St. Bartholomew's Hospital in London, from where he graduated with distinction in 1978. Following a series of house jobs at London hospitals, he won a fellowship from the UK National Medical Council in 1984 to study renal medicine at the Nuffield Department of Medicine at the University of Oxford. In 1987, Ratcliffe was hired as a clinical lecturer in the department.

Having published a handful of case studies, he was now eager dive deeper into scientific research. After a false start or two, he decided to explore the body's ability to sense and respond to subtle changes in oxygen levels, a capability in which the kidneys were thought to play a central role. Ratcliffe began by exploring the organ's production of erythropoietin (EPO), a hormone (first cloned by Ludwig researchers) that stimulates the production of oxygen-carrying red blood cells.

EPO production is exquisitely attuned to oxygen levels in the body, so it was widely believed that some factor X that regulates the expression of the EPO gene would be the body's oxygen sensor. To find it, Ratcliffe and many other researchers, including Gregg Semenza, were looking for a DNA sequence—a regulatory element—that boosts EPO production when switched on by the putative sensor.

Ratcliffe and a trainee nephrologist in his lab, Chris Pugh, described in 1991 a short DNA sequence near the EPO gene that did just that. But it soon became clear that their premise needed reexamining. Ratcliffe, Pugh and another nephrologist trainee in the lab, Patrick Maxwell, soon discovered that the hypoxia-responsive DNA element was active in all sorts of mammalian cells, not just those that produce EPO.

"We were so prejudiced that the oxygen sensor was specific for EPO that we were looking to identify the process by transferring it from an EPO-producing cell, which we thought would have it, to a non-EPO producing cell we believed would not," says Ratcliffe. "To our astonishment, we found the property wasn't private to the EPO producing cells. It was general. That experiment transformed my life. It brought me into contact with cancer research and other types of biology."

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Around the same time, Semenza reported his discovery of HIF-1α, a master regulator of gene expression that drives the hypoxia response of cells. He subsequently showed that its product combines with a standard-issue nuclear factor, HIF-1β, to switch on the gene expression that drives adaptations to hypoxia. By 1994, Ratcliffe and his colleagues had identified the first of the hundreds of non-EPO genes regulated by HIF-1α, and they turned out to encode metabolic enzymes—particularly those known to play a critical role in cancer metabolism, a finding confirmed by Semenza's group.

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Ratcliffe's team reported three years later that tumors engineered to be defective in HIF-1β had trouble growing in a mouse model, cementing the importance of hypoxic pathways in cancer. That this should be the case was not exactly a surprise. It was well known that the cores of tumors are often starved of oxygen and that hypoxia can drive drug resistance and metastasis.

The big breakthrough

The race was now on to find the factor that regulates HIF-1α—the primary oxygen sensor that would give every cell in the body the ability to respond directly and swiftly to that indispensable resource, oxygen.

A clue came from the Harvard laboratory of William Kaelin, who was studying von Hippel-Lindau syndrome, an inherited propensity for cancer that often manifests in the kidneys. Kaelin reported in 1996 that pVHL, a tumor suppressor protein mutated in the cancer, normally suppresses many hypoxia-related genes. The field had, meanwhile, identified three regions of HIF-1α crucial to the protein's function in hypoxia. These domains received some signal transmitted by the unknown oxygen-detector in cells.

Ratcliffe and his colleagues showed that the signal itself was atypical—that is, not conveyed by enzymes known as kinases that add a phosphate group to specific amino acids on proteins. They also began exploring what exactly pVHL was doing in the oxygen-sensing business, and reported in *Nature* in 1999 that when oxygen is abundant, the tumor suppressor interacts directly with HIF to target it for degradation.

Two years later, they reported in *Science* and the *EMBO* journal that pVHL recognizes two specific amino acids—proline residues—in HIF-1α that are independently chemically modified by the addition of an oxygen atom to create hydroxyproline. Kaelin and his colleagues simultaneously published similar findings. That same year, Ratcliffe and his colleagues, now including a collaboration with an Oxford chemist, Christopher Schofield, reported in *Cell* the identification of the enzymes that are responsible for these hydroxylations. These enzymes are dioxygenases, which absolutely require molecular oxygen (O_2) to function.

These were the long-sought oxygen sensors that link oxygen levels to hypoxic responses. When oxygen is abundant, the enzymes—known in humans as PHD-1,2 and 3—hydroxylate the HIFs, setting them up for pVHL binding and their subsequent degradation. When oxygen is scarce, they fail to hydroxylate the amino acids and the

Photo by Paul Wilkinson

HIFs are permitted to linger on and trigger the necessary cellular adaptations.

The use of hydroxylation to control these responses also represented a new mechanism of signaling within the cell. "Hydroxylation wasn't an unprecedented modification," of proteins, says Ratcliffe. "But as a signaling mechanism it was at the time unprecedented." The discovery of the oxygen sensing system in cells would enable new approaches to treating cancer—and many other ailments in which hypoxia plays a major role, from anemia to heart disease to wound healing.

Cancer's pathways

Over the next several years, Ratcliffe explored the biochemistry of HIF regulators and, with his colleague Christopher Schofield, began designing inhibitors of the family of enzymes that inhibit HIF as potential therapies. With others, his lab also showed that HIF-2, specifically, was a driver of clear cell renal carcinoma. This discovery led to the ongoing development of HIF-2 targeting drugs for that cancer by scientists at the University of Texas South Western and a biotechnology company.

In 2018, Ratcliffe—who is also director of the Target Discovery Institute at the University of Oxford, and clinical research director at The Francis Crick Institute in London—published with his colleagues a study in *EMBO Reports* mapping HIF binding across the genome.

Photo by Paul Wilkinson

Although the two HIFs recognize the same sequence of DNA, they showed that each activates distinct suites of genes in every cell type examined and cannot compensate for the loss of the other. This implies that each of the HIFs may be independently targeted to induce distinct therapeutic effects, much as HIF-2 is being specifically targeted in kidney cancer.

Yet how hypoxia pathways drive cancer progression, says Ratcliffe, remains mechanistically unclear. The hypoxia response alters almost every aspect of the cell's internal life, sparking—as their study showed—the expression of hundreds of genes and the activation of countless biochemical pathways. Further, experimental evidence suggests that some of those pathways drive malignancy, while others work in the opposite direction. In fact, HIF activation is inhibitory in some cancers. It thus seems likely that the cells of tumors in which HIF is activated need to modulate, or tune, the pathway, and that the cells which drive cancer are the products of an evolution that ultimately favors the pro-cancerous pathways while muting suppressive ones.

"Only when the mutations are right, pathways are right, the tissue context of the cell is right, and previous mutations have occurred that help set the stage—only then can that pathway switch be tolerated and promote cancer," says Ratcliffe. "I think this is a central principle restraining tumor development and a central issue that we have to understand if we're going to understand cancer." Ratcliffe is preparing now to examine his hypothesis using hypoxic signaling in renal cancer as a model.

Back to basics

The oxygen-sensing system discovered by Ratcliffe in 2001—in which oxygen levels are directly linked to the degradation or retention of proteins governing the hypoxic response—was initially thought to be unique to animal cells. But over the years parallel mechanisms of sensing and responding to oxygen levels were discovered in all the other kingdoms of life as well.

In plants, the sensing is executed by a family of enzymes known as plant cysteine oxidases (PCOs), which prime proteins for destruction in a different way. The existence of these and other such mechanisms of oxygen sensing got Ratcliffe wondering whether human cells might harbor alternative oxygen sensors. Evolution, after all, tends to favor redundancy in mission-critical processes, and oxygen sensing certainly falls into that category.

In 2016, while attending a meeting in Rome,

Ratcliffe got into a discussion on the matter with Francesco Licausi, a plant physiologist at the University of Pisa. They wondered whether the plant system of oxygen sensing might also be present in human cells and what would happen if plant oxygen sensors, known as PCOs, were inserted into human cells. Would these plant sensors still be able to regulate hypoxic responses in their new homes, exposing an unknown mechanism of cellular oxygen sensing? The pair decided to find out when they got back to their labs.

The researchers began by constructing a readout for the proposed experiment: a fusion protein built from the oxygen-sensitive part of a PCO target named RAP2.12 and a fluorescent protein. They then engineered cancer cells to stably express the fusion protein, and exposed them to hypoxic conditions. To their surprise, the hypoxic cancer cells glowed considerably longer than their oxygenated counterparts, even though they hadn't yet been engineered to express PCOs.

"That told us that something in the human cell was working on the artificial plant protein," Ratcliffe explains. A search of the genome revealed that the enzyme, cysteamine (2-aminoethanethiol) dioxygenase, or ADO, was one of two proteins in human cells that resembles PCOs and would fit the bill. Notably, the similarities between ADO and the PCOs indicate that this mechanism of oxygen sensing arose several hundred million years ago in some primitive, cellular progenitor of both the plant and animal kingdoms. Remarkably, the researchers showed that PCOs would substitute for ADO in human cells and insertion of human ADO would revive plants that were deficient in PCOs.

They also identified three of ADO's protein targets and showed that the ADO system and the HIF system work on different timescales. Since ADO can alter other signaling proteins directly, the sensor exerts its effects in the range of minutes to hours. HIFs, by contrast, exert their effects over hours to days because they drive the expression of genes whose products then drive hypoxic signaling cascades.

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This is physiologically relevant. "For example, the constriction of blood vessels in response to hypoxia has to occur very rapidly," says Ratcliffe, "whereas acclimating the body to reduced oxygen at higher altitudes can occur more slowly." Given the centrality of oxygen to biological processes, the newly discovered system of oxygen sensing, like that of the HIFs, is likely to play a role in diseases like cancer as well.

Ratcliffe suspects there are more oxygen sensing systems to be found, including a type that exerts its effects in a matter of seconds. If so, it's probably fair to say he's qualified to find them.