Profile of Gregg L. Semenza

Hundreds of millions of years ago, mitochondria acquired the ability to produce energy in the form of ATP by cleaving carbohydrate molecules with oxygen. Ever since, animals have been increasing in cells and size. To say that the development of life on our planet, especially metazoans—the diverse species of the animal kingdom—relied on this ability to harness oxygen may be an understatement.

"Without oxygen for 5 minutes, you're dead," sums up Gregg L. Semenza, professor of pediatrics at The Johns Hopkins University (Baltimore). He has spent his career using genetics and molecular biology to study various aspects of how living systems use and regulate oxygen. He discovered that one transcription factor, hypoxia inducible factor-1 (HIF-1), directs a vast program of responses to oxygen from the formation of the cardiovascular system in embryos to a cell's specific responses to low-oxygen conditions. For this work, he was elected to the National Academy of Sciences in 2008.

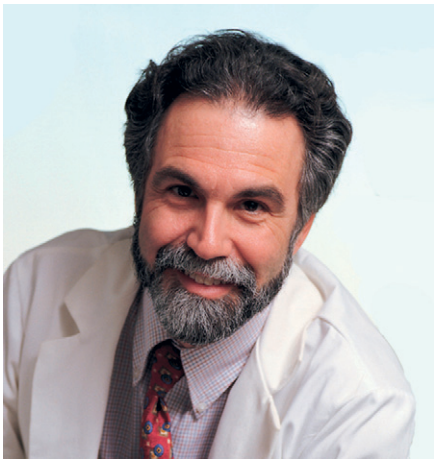
"The range of biological processes involved here is just staggering," Semenza says. In fact, his research demonstrates that HIF-1 may regulate 5% or more of the human genome, including a direct involvement in mitochondrial respiration (1–3). HIF-1 has been implicated in disease processes too, and Semenza and others have shown that HIF-1 is overexpressed in a majority of human cancers.

Semenza's Inaugural Article details a comprehensive search for drugs that inhibit HIF-1 and, thus, may interfere with the ability of certain cancers to metastasize (4). Cardiac glycosides, typically used to treat congestive heart failure and cardiac arrhythmias, emerged as the most promising. In particular, the drug digoxin increased the latency period of malignancies and decreased tumor growth.

Geneticist to Pediatrician

Born in Flushing in the New York City borough of Queens, Semenza grew up north of there in Westchester County. The eldest of five siblings, he attended Sleepy Hollow High School in North Tarrytown, where his biology teacher, Rose Nelson, inspired him to study the sciences. "She transmitted the wonder of biology and the excitement of understanding the subject at fundamental levels," says Semenza.

The son of a psychotherapist and an elementary school teacher, Semenza's first exposure to scientific research came during a summer he spent at the renowned Boyce Thompson Institute for Plant Research located in Yonkers, NY, at the time. After graduating from high school



Gregg L. Semenza.

in 1974, Semenza attended Harvard University (Cambridge, MA) with the intention of pursuing a career in basic genetic research. Not long after he began college, however, a family friend had a child born with Down syndrome.

"That experience," he recalls, "spurred me toward medical genetics and into applying for MD/PhD programs. It was a big switch for me."

He got his first experience in the field as an undergraduate in the laboratory of pediatric geneticist Park Gerald at Children's Hospital Boston. There, he worked on somatic hybrids between mouse and human cells to map genes on chromosome 21, the extra chromosome present in people with Down syndrome.

After graduating from Harvard, Semenza joined the MD/PhD program at the University of Pennsylvania (Philadelphia) and began studying the recessive genetic disorder, β-thalassemia. The disorder is one of a family of diseases that occurs when red blood cells do not produce enough of the individual proteins that make up hemoglobin, the main oxygen transporter in blood. In fact, it was the first family of diseases in which a DNA mutation was definitively identified as the cause.

For his doctoral research, Semenza worked in the laboratory of Elias Schwartz and Saul Surrey at the Children's Hospital of Philadelphia to sequence the β-globin gene of an Albanian family with β-thalassemia. His work identified a rare silent carrier allele without a mutation in the coding or flanking regions of the β-globin gene (5).

As he worked his way through his graduate and medical studies, Semenza quickly realized that specializing in pediatrics would best prepare him for a career in medical genetics. "Most genetic disorders, conditions like Down syndrome and dwarfism, are apparent at birth, so a lot of the patients are children," Semenza says. "To understand these diseases, you have to understand human development, which is what pediatricians are trained to do."

After finishing both advanced degrees, Semenza completed his medical training with an intensive internship and residency in pediatrics at Duke University (Durham, NC) in 1986. From there, he accepted a postdoctoral fellowship in medical genetics at Johns Hopkins, where he has been ever since. "Hopkins is a unique place," he says. "There are tremendously talented people here all working in a very collegial atmosphere—this is a combination that you won't find anywhere else."

Semenza serves as the founding director of the Vascular Program at the Johns Hopkins Institute for Cell Engineering. His wife, Laura Kasch-Semenza, whom he met at Hopkins, runs a genotyping core facility at the university.

Courtesy Leads to Breakthrough

At Hopkins, Semenza gained access to leaders in the field of thalassemia research. "Doctors Haig Kazazian and Stylianos Antonarakis were then at Hopkins," he says. "They drove the field—dissecting different types of mutations that occur in patients and understanding how these changes interfered with gene expression."

As it turned out, thalassemia did not hold anyone's attention long. Semenza spent his first year in Baltimore attending to medical genetics patients and working nights in the pediatric emergency room. And by the late 1980s, Kazazian's and Antonarakis's research teams had moved on from studying thalassemia genes to mapping the genes involved in hemophilia, such as factor VIII. This genetic element encodes a clotting factor deficient in hemophilia A, the most common form of the hereditary disease. Semenza proposed a research project in which he would express the human factor VIII gene in mice by creating a transgenic animal, a technique that had been in use only for a few years.

To acquire a genetic clone of factor VIII, Semenza contacted researchers at the Genetics Institute, a biotechnology company in Cambridge, MA. In addition to the hemophilia clotting factor, scientists there had also cloned the gene for erythropoietin (EPO), a hormone that controls the production of red blood cells.

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 19579 in issue 50 of volume 105.

They suggested that Semenza might find this gene interesting to study, so they sent it along as well. As it turned out, this simple act dramatically altered Semenza's research course.

In collaboration with stem cell pioneer John Gearhart at Johns Hopkins, Semenza succeeded in creating transgenic mice that expressed human EPO. "The mice made more red blood cells than normal," explains Semenza. "So we not only showed that we could express the human EPO gene in mice but that it could have a biological effect (6)."

Soon after, the National Institutes of Health sent out a request for applications that sought to fund research into the regulation of the EPO gene. Semenza applied, and after winning the grant, he quickly found himself in the enviable position of being a postdoc with the research support of a faculty member. Around the same time, in 1989, the Lucille P. Markey Charitable Trust, an organization that outspent the Howard Hughes Medical Institute in its 13 years of existence, also awarded him funding.

"I was extremely fortunate to essentially have two major grants before I started my faculty position," he says. "It was due to great mentors—Haig Kazazian, Stylianos Antonarakis, John Gearhart, and Victor McKusick—helping me all along the way."

Tackling a Tricky Transcription Factor

With his funding secure, Semenza continued studying red blood cell regulation. Certain cells had been shown to produce more EPO in low-oxygen or hypoxic conditions as part of a well-regulated oxygen homeostasis mechanism. He set out to find the genetic controls that triggered this reaction.

He started by making fragments of the noncoding regions of the EPO gene and cloning them into reporter constructs. After much genetic "cutting and pasting," he identified a 33-nucleotide section downstream of the EPO gene that was the smallest element that induced EPO during hypoxia (7). Next, Semenza set his sights on finding the protein, a transcription factor, that bound to this EPO gene regulatory sequence and increased production of the hormone.

The identification of transcription factors can be difficult. Cellular, physiological, and chemical conditions all affect whether any particular protein binds a segment of DNA. According to Semenza, "you can try many different conditions, but if you get a negative result, you never know if you're getting closer or further away."

In fact, he came precariously close to missing the discovery of what would turn out to be HIF-1. "By this time," says Semenza, "my first postdoc had joined the lab, a fabulous scientist named Guang Wang. He was conducting these binding studies and not having any luck. One day, as I was flipping through his negative results, I held up an image from a gel-shift assay and saw a faint band. And there it was: HIF-1. Within a couple of weeks, Guang had turned that faint band into a screaming result just by optimizing the conditions."

Semenza and Wang began purifying and characterizing the finicky transcription factor. In a paper published in PNAS (8), his group demonstrated that two proteins, HIF-1α and HIF-1β, composed the holoprotein. Levels of the HIF-1α subunit increased dramatically under hypoxic conditions but rapidly decreased when oxygen levels returned to normal.

Paradigm of Regulation

Semenza's group searched for other genes that became activated in low-oxygen conditions to see whether HIF-1 regulated them as well. They soon found that HIF-1 also bound to and regulated a promoter region of vascular endothelial growth factor (VEGF), a signaling molecule that earlier research showed promotes the formation of new blood vessels, and that HIF-1 is implicated in many cancers (9).

"The sequence that HIF-1 binds in the VEGF gene is almost identical to the sequence that it binds in the EPO gene," says Semenza "These studies created a paradigm of how to explore regulation of a target gene by HIF-1."

To extend this research, Semenza worked again with Gearhart to create mouse embryonic stem cells that lacked HIF-1α and, therefore, could not produce a functional transcription factor. Semenza found that, after the eighth day of gestation, when mouse embryos normally establish a functional circulatory system to distribute oxygen to an increasing number of cells, HIF-1α knockout embryos failed to continue developing and displayed major malformations, especially in the cardiovascular system (10). "In the absence of HIF-1," says Semenza, "there's a failure of the normal development of the heart, the blood vessels, and the blood —all three components of the circulatory system fail, and the embryos die."

This research, concluded in 1998, gave scientists a first glimpse at the importance and necessity of HIF-1. The transcription factor straddled two worlds: It contributed to the physiological development of the body's oxygen homeostasis mechanism by promoting the formation of the cardiovascular system, and, after birth, it helped this system adapt by increasing crucial proteins such as EPO and VEGF in low-oxygen conditions.

Semenza calls it "a beautiful system to ensure that every cell gets enough oxygen. Our work demonstrated that the distinction between development and physiology is a bit artificial. It's all part of the same process and, in this case, it's being driven by the same signal."

Linking HIF-1 to Cancers

Problems occur, however, when this system fails. Cancer, aging, and chronic illnesses interfere with the careful regulation of homeostasis, and HIF-1 has emerged as a critical player in common human diseases and a target for therapies.

At Johns Hopkins, Semenza found an enthusiastic colleague to collaborate on research into HIF-1's role in carcinogenesis. "Oncologist Jonathan Simons immediately recognized the importance of the transcription factor in what he was studying," says Semenza. "Simons said, 'HIF-1 is one of the most important factors in cancer,' in a much more fanatical way than I did at that time."

An investigation into 19 different tumor types, including colon, breast, lung, and skin cancer, would prove Simons right. Thirteen of these malignancies overexpressed HIF-1α. In addition, although only one-third of primary breast cancer tumors increased their production of the transcription factor, nearly 70% of breast cancer metastases did (11).

"We know that cancers have hypoxic areas where the tumor does not get enough oxygen," Semenza says. "In fact, tumors with the lowest amounts of oxygen are most likely to metastasize and kill the patient. So, we started looking at the mechanism by which HIF-1 overexpression contributes to different cancers."

Regulation by Hydroxylation

In 2001, researchers made big strides in understanding how cells regulate HIF-1. First, a trio of scientists—William Kaelin at Harvard, Frank Lee at the University of Pennsylvania, and Peter Ratcliffe at the University of Oxford (Oxford, England)—discovered the pathway by which molecular oxygen regulated the transcription factor (12–14). After the discovery by Ratcliffe's laboratory that, in healthy cells under normal oxygen conditions, the von Hippel–Lindau protein (VHL) marks HIF-1α for degradation, the researchers discovered enzymes that use oxygen to hydroxylate HIF-1α on key proline residues. This enzymatic action allows VHL to bind the transcription factor and target it for degradation by proteasomes. In low-oxygen conditions, these prolyl hydroxylases cease functioning, HIF-1α is stabilized, and the expression of proteins such as EPO and VEGF increases. Semenza calls the results "a real breakthrough in terms of understanding oxygen sensing."

Around the same time, a postdoc in Semenza's laboratory, Connor Mahon,

identified a protein that prevented HIF-1α from binding coactivators it needed to initiate transcription (15). Murray Whitelaw's laboratory at Adelaide University (Adelaide, Australia) subsequently demonstrated that this protein, which was named FIH-1 (factor inhibiting HIF-1), also hydroxylates the transcription factor by using cellular oxygen, but on an asparagine rather than on a proline residue (16).

Thus, the presence of oxygen has two different effects on HIF-1α: Proline hydroxylation allows VHL to bind, which sends the protein to proteasomes, and asparagine hydroxylation prevents coactivators from binding, thereby blocking the transcription factor from increasing the production of EPO and VEGF.

According to Semenza, "there were many speculative models about how hypoxia regulated HIF-1, which all turned out to be wrong. Regulation by proline or asparagine hydroxylation was not one of the early models that were bandied about, so it was a big advance in the field to identify both of these mechanisms."

Common Cancer Component

Cancerous cells, however, inactivate tumor suppressor molecules such as VHL, thereby disrupting hydroxylation pathways. This interference mimics low-oxygen conditions and leads to HIF-1α stabilization and the unregulated growth of malignancies. "This turns out to be a very important mechanism by which HIF-1 gets turned on in cancer cells," says Semenza.

Breast cancer research conducted by his group shed light on another mechanism by which HIF-1 contributed to carcinogenesis. Overexpression of the *HER2/neu* gene, which encodes a growth factor receptor, in breast cancer strongly correlates with VEGF up-regulation, aggressive metastasis, and resistance to chemotherapy. Semenza's work demonstrated that HER2 signaling increases HIF-1α protein synthesis, rather than stabilizing the protein (17). HER2's effect on the transcription factor occurred independent of hypoxia.

The outcome led Semenza and other scientists to conclude that HIF-1 has multiple roles in the formation of tumors as it does in development: It can be induced by genetic alterations and low-oxygen conditions. "The combination of these pathways leads to very high HIF-1 levels, which we now believe to be a final common component through which many of these genetic changes affect cancer biology," he says.

Back to the Clinic

With the wealth of knowledge he has accumulated studying the master transcription factor, Semenza has decided to focus his current research on finding ways to improve the treatment of diseases in which HIF-1 plays a role.

"One of the most exciting things about studying HIF-1 for me is following the entire range of biological processes," he says. "You can start with the most basic, fundamental questions like 'How does the cell sense the concentration of oxygen and respond to changes,' all the way to the most applied, 'How do we develop new treatments for cancer and cardiovascular disease?'"

"By virtue of my training," he continues, "I can bridge between the basic science and the clinical work. I feel that this is the most useful way for me to approach these problems, and it's what I find most satisfying."

In his Inaugural Article (4), Semenza and colleagues screened more than 3,000 drugs that are used either as treatments or are in clinical trials to identify HIF-1 inhibitors. "This was crucial," he says, "because we weren't just using a library of chemical compounds—many of which may be toxic to patients." They uncovered a class of therapeutics, cardiac glycosides, that specifically inhibited HIF-1α protein synthesis in cancer cells. One drug in particular, digoxin, potently blocked tumor growth in mice in Semenza's study. Clinical trials that use the glycoside for cancer therapy soon will be underway.

Semenza's group is also investigating the role that HIF-1 plays in age-related diseases. As the body gets older and vessels constrict, blood supply decreases to the extremities as well as to vital organs. This constriction can lead to fatal ischemia. Semenza's research showed that the loss of HIF-1 as a result of aging impairs the body's ability to recover from peripheral arterial disease and ischemia. But when Semenza's team administered to mice a viral vector carrying the HIF-1 gene, it helped the mice recover, paving the way for the potential use of the transcription factor in gene therapy (18).

In recognition of his work, the Society for Pediatric Research awarded Semenza its E. Mead Johnson Award. The society has given the award since 1939 to celebrate outstanding clinical and research accomplishments in pediatrics. Semenza also received a 2010 Canada Gairdner International Award, which is given annually to individuals from a diversity of fields for outstanding discoveries or contributions to medical science.

Semenza remains unfazed by his awards and the direction his research has taken. "It's not like I came up with a fantastic idea to launch some new area of research," he says. "I've just continued to follow what we've been doing and to try to understand at a greater depth the basic science, and then to try to move toward clinical applications. It's all very incremental."

"I just find what we're doing so fascinating and so enjoyable that it's a pleasure to come to work each day and to work on these projects. I feel remarkably lucky," he says.

Farooq Ahmed, *Freelance Science Writer*

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