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Cell

Making Sense of the Unexpected

The 2016 Albert Lasker Basic Medical Research Award is being awarded to Greg Semenza, William Kaelin, and Peter Ratcliffe for discovery of the pathway by which human and animal cells sense and adapt to changes in oxygen availability—an essential requirement for survival. Bill and Peter joined *Cell* editor João Monteiro in an informal conversation about science, medicine, designing experiments, and training the next generation.

William G Kaelin
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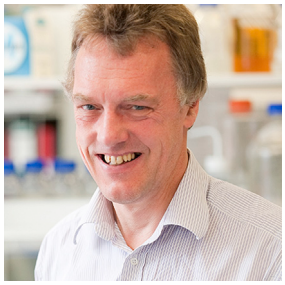

Peter Ratcliffe
Nuffield Department of Medicine, University of Oxford

Joao Monteiro: Do you remember how you two first met?

William Kaelin: I saw a poster in a meeting in Paris, presented by Patrick Maxwell, one of Peter's students at the time. He was kind enough to tell me that Peter had work in press related to deregulation of HIF and the effect of the VHL gene in kidney cancer cells and that I should get to know his mentor. I think we first started communicating within about a year of that. Does that sound about right, Peter?

Peter Ratcliffe: That's about right, Bill. Patrick was in fact one of a series of trainee nephrologists, including Chris Pugh and others, who joined the laboratory and made a tremendous impact. The work was in progress, but it was not in press. That took quite a while, actually.

WK: Patrick and I understood that the next questions would be related to how the interaction between VHL and HIF was regulated by oxygen, and he said I should really be speaking with Peter. I think I reached out to Peter probably sometime in late 2000—I'm guessing when we started to have some of the data that led to our *Science* paper in 2001.

PR: That's right. We both knew we had something interesting, but we didn't know that we had pretty well exactly the same thing until those submissions to *Science*. There were other sites of interaction, which were slightly puzzling but came clear later. It's always reassuring when a well-respected colleague has the same results as one's own lab.

JM: Let me ask you, Peter, how did you get involved with these questions?

PR: It's a long and torturous journey. I got in from the oxygen-sensing angle, which was a quest for me in slightly different ways from the outset. I trained as a nephrologist and was interested in why the kidney was susceptible to shock. Clinically one often sees kidney injury in people with low blood pressure. It was believed that this had something to do with the unusual countercurrent circulation of the kidney, which creates a very low oxygen tension in the interior of the organ, though this has never been fully understood. From there, I became interested in understanding why the kidney can make the hormone erythropoietin in response to reduced oxygen availability, but not as a response to reduced blood flow. I thought it might have something to do with the circulation that I just spoke about, but we didn't resolve that one either. To my knowledge it isn't understood to this day. The next transition was to consider the oxygen-sensing process itself, which we believed was very special and exclusive to the kidney. That's how I got into the field, rather roundabout. All that was in the 1980s.

JM: When did you realize that oxygen sensing and the HIF pathway would become such a central signaling pathway, important in so many systems and on so many different cell types?

PR: Well, I can remember pretty well to the moment the radiograph was coming out of the developer. As I was saying, it was believed that oxygen sensing was a special property of particular tissues and cells. We were working on hepatoma cells, which were sensitive to oxygen tension, and I wanted to establish a system to study how this process was regulated.

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Because we had this strong prejudice that oxygen sensing was not widespread, we chose COS cells that don't make erythropoietin and (so we thought) shouldn't be able to sense oxygen. They make a very good system for expression cloning, and that's what we wanted them for: to provide a recipient cell. When we transfected the cells with the reporter gene, to my surprise, the results suggested that oxygen sensing was happening in COS cells too. I was initially irritated when I saw the results because I had a planned set of experiments, which was obviously disabled by these findings. However, the more we thought about it, the more we realized that the consequences of that result might be rather profound.

WK: I think that this beautifully illustrates two points that I'm sure Peter would agree with me on. The first is the point I try to drum home with students all the time, which is how often important discoveries start with unexpected behavior with one of the controls. The second, as I once heard a Nobel Prize winner say, is that a surprisingly high number of what would have been great discoveries probably wind up in the waste paper basket because they didn't fulfill people's biases. When things don't fulfill your biases, sometimes they tend to be ignored or discarded. But retrospectively, you realize how important the observation was. I thought that was a great vignette Peter just described.

PR: That's my experience, and I agree with you. I think many of the most important results in my lab initially irritated me.

WK: I have a saying, which is probably an over-simplification, that engineers live for the expected results, and scientists live for the unexpected results.

JM: You both also are physician-scientists. Do you think that this background influenced the way that you do science? Did it make a difference regarding the type of questions that you asked or the way that you approached them?

PR: I thought it made me more forceful. I was 35 years old when I started this project. I think that, as a physician, you get more confident about dealing with unknowns. I always found it helpful not to be too shy to ring up for advice and push until you've got the advice you needed. A bit of that was drawn from high-pressure clinical medicine. You have to get things done there, you have to make decisions. But there is one essential difference between the two. In the clinic, if you don't know what to do, do nothing. In the lab, if you don't know what to do, do something. In the clinic, the experiment is ongoing before your eyes, so you just need to wait and more information will come. Whereas in the lab, of course, that can't possibly happen.

JM: What about you, Bill?

WK: Part of my answer relates to your first question, about how I got into the field of oxygen sensing. When I was a young physician, I was pretty sure I was going to be a practicing clinician. I had actually done a so-called chief residency at Johns Hopkins. Chief residents love rare eponymous syndromes like von Hippel-Lindau [VHL] syndrome because they can use them to assert their authority on rounds. If a trainee steps out of line, they can embarrass them by asking them questions about such rare entities. Clinicians also tend to memorize differential diagnoses—all the possible causes for any symptom or sign you might encounter on the wards. When the Hippel-Lindau gene was cloned, I knew what cancers it was linked to, such as kidney cancer. I knew that those tumors were very rich in blood vessels, so I hoped that studying VHL would teach us something about kidney cancer. If not, it would at least teach us something about how the angiogenesis is controlled. Q1

Another obscure fact about VHL-associated tumors is that they occasionally cause the body to produce too many red blood cells. What angiogenesis and erythropoiesis have in common is that they're normally induced by hypoxia. It seemed to me that the tumors in VHL disease were behaving as if they constantly thought they were hypoxic and were sending out the distress signals that would normally be induced by hypoxia. This experiment of nature, if you will, could help us begin to understand the molecular circuitry of oxygen sensing.

JM: Looking toward the future, where do you think that the oxygen-sensing field is going? What questions are exciting you right now?

WK: We continue to be interested in whether there are settings where pharmacologically modulating the HIF pathway would be beneficial and could be exploited for therapeutic purposes. As you may know, there are drugs that target the HIF pathway that have advanced to phase III trials for treating anemia. I think the pre-clinical data are suggestive that, in certain diseases such as in heart attack and stroke, manipulating the HIF pathway pharmacologically might also be helpful. Those would be situations where you would want to ramp up the HIF response. Conversely, in a variety of cancers, we wonder whether dampening the HIF response might be a useful thing to do. I think that's going to be particularly true for cancers linked to loss of VHL, for instance. There are a lot of pharmacological opportunities to be explored.

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From a more basic or fundamental level, it remains to be seen how many other enzymes in the cell are regulated by oxygen, whether there are other proteins that, like HIF, are prolyl hydroxylated or undergo oxygen-dependent modification. There is also good reason to think that VHL has functions other than modulating or regulating HIF. There have been hints of HIF-independent functions of VHL, but there are a lot of unknowns there.

JM: What about you, Peter? What are you interested in these days?

PR: Pretty much the same thing but with a somewhat different bias. We now work quite closely with Chris Schofield, who is a chemist and is designing novel inhibitors. We're interested in whether different inhibitors can meet different medical challenges and believe that with sufficient investment this will be possible. I admire the people going forward with phase III trials for anemia. It looks really promising, and we hope it works. But we think that more inhibitors, more pharmacology, and lots more experimental medicine are required to tease out what is possible. A drug for ischemia—for low or inadequate blood flow, for instance—would be a terrific addition to the pharmacopeia.

Just as Bill said, we're also interested in other forms of hydroxylation, their regulation. And also in cancer. I feel it might be worth debating whether we think that deregulation of the HIF pathway causes cancer. I suspect that both pro- and anti-tumorigenic effects of the HIF switch require re-balancing or fine tuning as cancer develops.

WK: I think there are quite a few review articles that would suggest, largely based on guilt by association, that HIF universally promotes tumor growth and we should be developing HIF inhibitors. That's based largely on the fact that upregulation of HIF is often associated with a bad prognosis, but that could be because aggressive tumors outgrow their blood supplies and could become hypoxic and upregulate HIF. It's certainly true that HIF activates genes involved in tumor growth, but as Peter just indicated, there's really far more nuance than that.

The bottom line is here, I think context is going to matter and we're going to have to figure out, probably on a cancer-by-cancer basis, when HIF is largely pro-tumorigenic, anti-tumorigenic, or neither. Which HIF paralog is involved, in what stage, and so on. I think there's mounting evidence that, in some settings, HIF might actually be anti-tumorigenic or might simply be window dressing and have nothing to do with the transformed phenotype whatsoever. I think that we will have to tease this apart before we rationally design or use HIF inhibitors in the clinic.

JM: Switching gears a bit, what's your approach to training young scientists? Has it changed in the past 20 years?

WK: By now, you've probably figured out that Peter and I are almost twin sons of different mothers. I think we're not going to disagree on too many things here. Getting back to the point we just discussed, I try to emphasize to people that the power of the experiment usually lies in the thoughtfulness of the negative and the positive controls. We try to make sure that, when we put together papers, you can see the positive and the negative controls. We also try, whenever possible, to provide corroborating lines of evidence for our conclusions.

Another saying I have is that there are two kinds of scientists. There is the scientist whose great fear in life is being second, and there is the scientist whose greatest fear in life is being wrong. I try to tell people that, over the course of time, I'd rather we were in the second camp. So we may get second place now and then, but let's try to make sure that what we publish is going to be correct now, correct in 10 years, and correct in 100 years. That doesn't mean that, on further review, with the benefit of time, additional interpretations won't arise. But at least the experiments themselves will have been well conducted and well controlled. The final thing I try to impress upon my trainees is, again, another old saying: "It's as hard to work on an uninteresting and unimportant problem as it is to work on an interesting and important problem." So I push my trainees to try to identify questions that we agree are interesting and important and that will move the field forward.

JM: Great. Peter, I imagine that you agree with most of that. Any differences on the way you run your lab?

PR: Sure. There is an issue that is quite interesting in Bill's comments that speaks to your perceptions of how you control experiments, where you set the bar to accept that a result is true. There's an order to these things as well as a question of security. You first hunt an interesting result, and then you control it to make sure that it is correct. But first you hunt it. Otherwise, people waste a lot of time setting up controls for experiments that are never going to work anyway. There's more to it than simple care. There's sort of an art to balancing how aggressively you make the first observations and then how brutally you control it. We're both clinicians, and I've been an active clinician a long time. I tend to take the same approach to running my lab and the decision-making process. What is the prior probability of this all being true, and what is the post-experimental probability of it being true?

That means that I'm integrating the "security" of all sorts of different types of data. Not every scientist uses that. I have worked with people who take experimental results in isolation at face value. Of course, these people are much less constrained by prejudice. They're perhaps, you could argue, more likely to discover things, but they're also more likely to make mistakes, as they sometimes disregard a lot of data when they come to a final conclusion. Actually making accurate diagnoses in the clinic and accurate interpretation of laboratory results are not dissimilar processes. They both require considering all manner of possibilities and then great care in coming to a conclusion. I try to teach this type of decision making, as it impinges on many different things.