



Patrick Moore and Yuan Chang happened upon a treasure trove for tumor biologists.

ON THE HUNT FOR A PATHOGEN, THEY
FOUND MUCH MORE THAN ANYONE EXPECTED
BY CHUCK STARESINIC

PIRATED GENES

Yuan Chang distinctly remembers the moment in 1981 when, during her orientation to medical school at the University of Utah, a second-year student strode to the front of the room. He was there ostensibly as a member of Physicians for Social Responsibility, but the presentation she remembers would have served equally well for a group called, say, Physicians for Unpredictable Behavior. He didn't begin with "Hello." He didn't mention his name. He didn't even say, "I'm here to tell you about Physicians for Social Responsibility." He seized the microphone and yelled, "THERMONUCLEAR WAR!"

And that was the first time Yuan Chang ever laid eyes on Patrick Moore. "What a weird duck!" she thought to herself, somewhat aghast. "How bizarre!"

PHOTOGRAPHY | C.E. MITCHELL

Perhaps in spite of this first impression, Chang soon got to know Moore in her histology class, where he was a teaching assistant. He was a set of walking contradictions and idiosyncrasies, she learned: a high school dropout who read *The New England Journal of Medicine* and frequently reminded her that she should read it, too. He plied her with journal articles, one of which made a lasting impression on both of them.

It was from the *Morbidity and Mortality Weekly Report*, a publication of the Centers for Disease Control and Prevention (CDC). (“Nobody else in medical school was reading the *MMWR*,” Chang points out today with a sideways glance at Moore.) A large number of homosexual men in New York and California had inexplicably developed aggressive and rapidly fatal cases of what had always been an exceedingly rare and nonaggressive cancer—Kaposi’s sarcoma (KS). One physician commented that several of these men had severe defects in their immune systems.

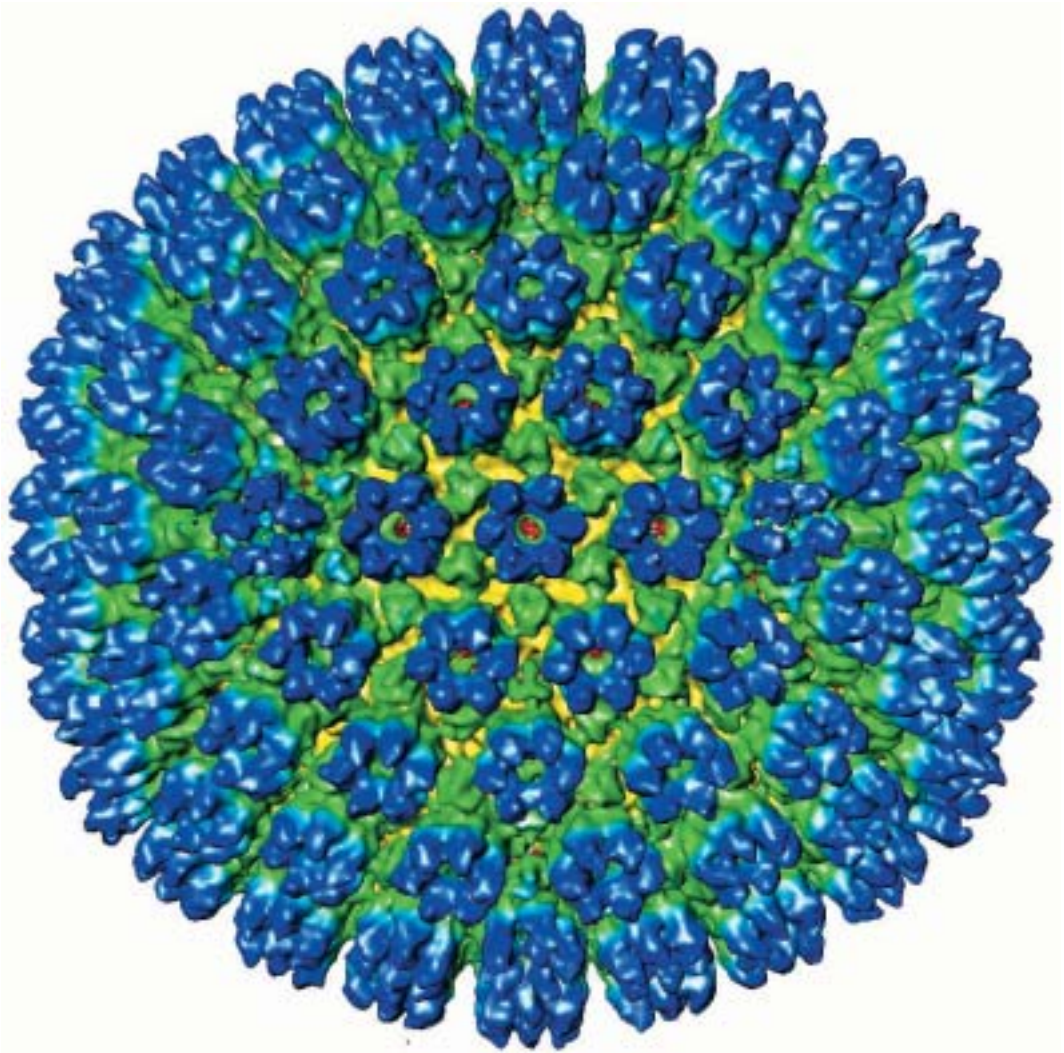
“This is really interesting,” Moore said to Chang when he gave her the article. “You ought to keep an eye on this. This is going to be big.”

The story was not big; it was colossal. It marked the emergence of the most devastating new disease of the century—AIDS. And though HIV would be identified within four years, finding a causative agent for KS—the leading malignancy in AIDS patients and the most common cancer in sub-Saharan Africa—would thwart the best efforts of laboratories around the world until 1994. That year two newcomers working on a shoestring, Chang and Moore, made the discovery that had eluded so many.

In the early days of the AIDS crisis, large numbers of patients were reported with KS as well as another previously rare condition, pneumocystis pneumonia. The latter was known to be the result of an infection, obviously made possible by the weakened immune system. But what was causing the KS?

The idea that viruses could cause human cancers was not completely new, but it was just beginning to be accepted. A virus called HTLV-1 was linked to leukemia only in 1980, human papilloma viruses to cervical cancer in 1983.

Throughout the 1980s and into the 1990s, articles regularly referred to the infectious etiology of KS. The disease appeared in patients whose immune systems were down, such as AIDS patients and transplant recipients. But not all such patients were at equal risk for developing KS. Those who developed AIDS



The virus KSHV pirates human genes. ABOVE: These are three-dimensional computer reconstructions of KSHV. KSHV is shown as a spherical capsid (coating) of interlocking proteins (left). The image on the right depicts a cross section. The red material represents internal components such as scaffolding proteins, amid which are the virus’ genes, including those pirated from the human genome.

following blood transfusions and sharing of needles rarely developed KS, while homosexual men in these groups, and especially those with a history of multiple sex partners and sexually transmitted diseases, had a greater than 50 percent likelihood of developing KS, notes Moore. It was as if a “WANTED” poster with no photograph had been hung in the town square. The profile was of an infectious agent that was sexually transmitted, more so in homosexuals than in heterosexuals.

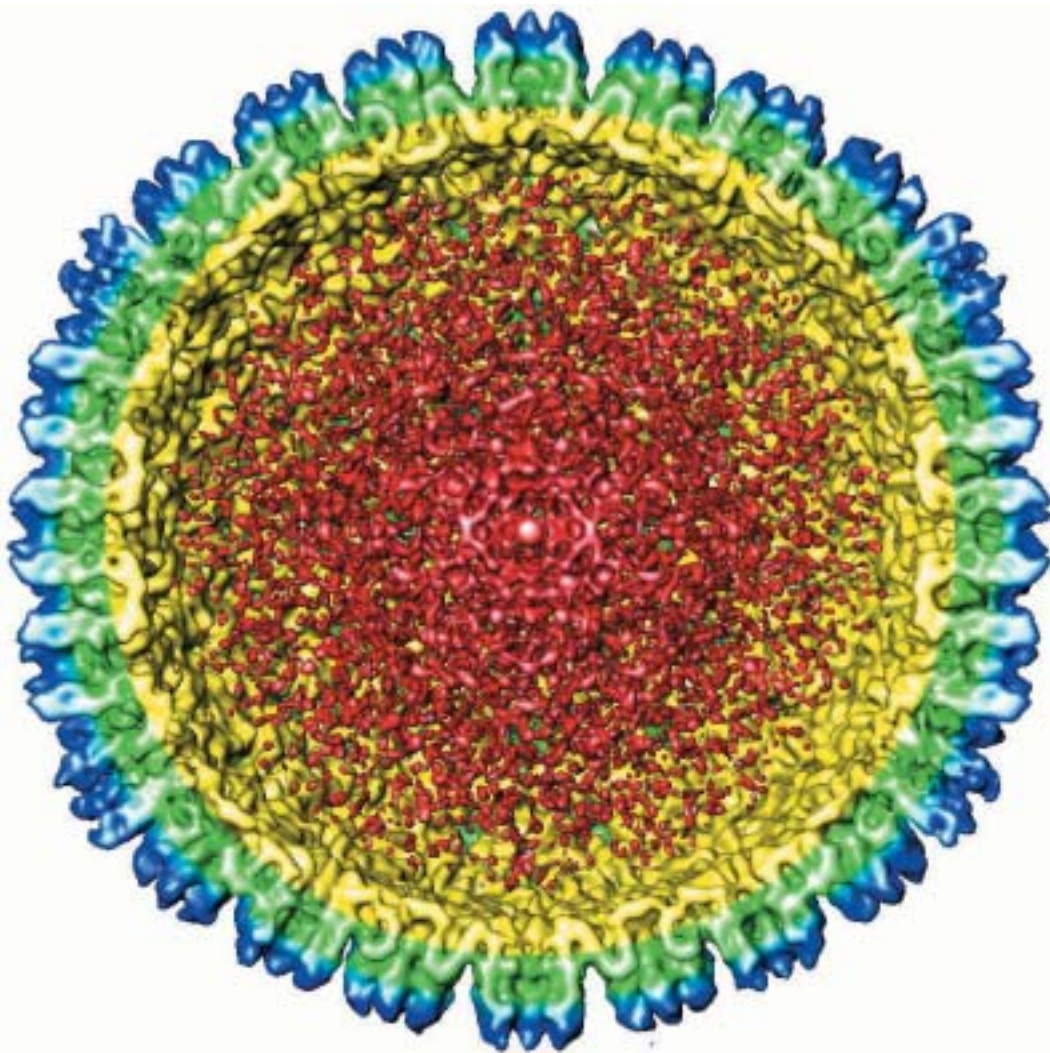
But after a decade or more of intense investigation into KS by some of the most prominent virology labs in the world, nothing turned up. Nothing conclusive was cultured or identified. A host of alternative theories was proposed, and the search for a pathogen languished. No one was looking to Chang and Moore for the answer to what was causing KS. No one, that is, except Chang and Moore.

Moore, an epidemiologist by then, had become obsessed by the search for new pathogens. His friend Tony Marfin remembers meeting Moore in 1989, when the two

were at the school of public health at the University of California, Berkeley: “Pat was talking about [KS] then. He was talking about methods that he could use to identify an infectious agent as being the cause of KS.”

Marfin later worked with Moore at the CDC in Fort Collins, Colo., and in refugee camps in Somalia and Nepal, where Moore continued to speculate about KS. Marfin laughs now, saying, “You know, the nature of the molecular investigations for KS are not the kinds of things you need to think about or know about when you’re in those refugee health situations. But he was always thinking about it then and still contributing greatly to the ongoing stuff that we were doing in those places.”

Moore believed that the secret to identifying new pathogens was to develop the right tools. He remembers working in Nigeria during an unidentified disease outbreak when people suddenly began dying of hemorrhagic fever, which could be caused by a lot of things, including the Ebola virus and yellow fever. Everyone was con-



cerned, and samples were rushed off for tests. They were relying primarily on serologic assays, in which blood is tested for the presence of antibodies to specific viruses; they'd tested for Ebola and yellow fever. When the results came back "inconclusive," the team thought they had a new agent on their hands. Waiting for test results in the middle of an unexplained outbreak was a vivid learning experience for Moore. It showed him that epidemiologists needed a means of diagnosing a viral infection without having any preconceived notions of what the virus was. In the end, the virus was yellow fever, with atypical characteristics that made it difficult to identify, but the lesson remained.

Moore had an ideal intellectual ally and foil in Chang. The two were married in 1989. He was the epidemiologist preoccupied with the cause of KS. Chang was the clinical neuropathologist determined to come up with new techniques for identifying the causes of neurological disorders. She loved working with neurologists, neurosurgeons, and patients. With cancers in particular, she wanted to develop more powerful molecular techniques for studying them in the lab, techniques that would reveal diseases in finer detail. Visitors to their home frequently found themselves party to impromptu discussions around the dinner

table exploring new methods of pathogen discovery.

Chang was recruited to Columbia University in 1993 to start a position as a clinical neuropathologist. Moore began working for New York City's health department. But more than ever he was preoccupied with the mystery of KS. Over cups of coffee at a Broadway cafe, the two talked about using Chang's lab for exploring KS. For both, it was not a question of *whether* KS had an infectious agent. The question was, how could they discover the KS agent, which had resisted all attempts at culturing?

The breakthrough arose out of Moore's habitual reading of the literature. Early in 1993, an article from *Science* emerged from Chang's fax machine at Columbia with Moore's handwriting scrawled across the title page: "Yuan, Check this out." The title was, "Cloning the Difference Between Two Complex Genomes."

Chang still has a hard time believing what

happened next. It doesn't matter that it has been 10 years, and she and Moore are sitting in the lounge area of their laboratory in the new Hillman Cancer Center. Or that they have recently returned from the National Institutes of Health, where they were awarded the Charles S. Mott Prize, given by the General Motors Cancer Research Foundation, one of the most prestigious awards for cancer research. Just over a decade ago, they'd thought about KS a lot, but had never set out to find the causative agent in a laboratory experiment. Nevertheless, in six weeks, they had it. First try.

"We were really lucky"—Chang says while she almost gasps with her hand on her forehead, as if a decade hasn't been long enough to recover. Yet the process was "arduous," reports the neuropathologist who was used to getting results from an experiment in two or three days. The technique they used, called RDA, took six weeks. "This is a protocol that—my God—once you're in the middle of it, there's no way to really check whether you're on course or not," she says. "You have to go all the way through it to the very end in order to know whether you did it right."

In one shot, RDA revealed the pathogen. "It was amazing," says Chang. "We were just really lucky."

RDA, or representational difference analysis, was novel at the time. No one had yet used it to discover a new pathogen. Like a lot of breakthrough ideas, the underlying concept was simple: RDA is a way to draw out the differences between two samples of DNA; if there is a difference between KS tissue and healthy tissue from the same patient, that difference is likely to be the cause of the disease.

Chang likens the original problem to having two sets of the notoriously exhaustive, multivolume *Oxford English Dictionary* that are identical except for a few extra words hidden in one volume. Imagine how long it would take to compare the dictionaries and find those extra words. Finding the culprit in tissue samples containing enormous amounts of DNA was a similarly intractable problem. At least it seemed intractable. RDA draws out variant sequences of DNA and clones them, amplifying them so that the difference can be seen readily.

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When Chang and Moore compared KS tissue with nondiseased tissue from the same patient, the difference between the two was a set of totally unique, distinctly herpeslike DNA sequences. They'd found another human herpes virus, the eighth yet discovered. It was soon to be called HHV8, or alternatively KSHV.

Laboratories from San Francisco to London quickly confirmed Chang and Moore's findings. More than a few KS researchers wondered who the newcomers were and from where they had come.

As exciting as the discovery of KSHV was, Chang and Moore were prepared to eventually stop studying it and move on to something else. But that has not happened, and it won't happen anytime soon. In fact, though they continue to seek out new pathogens, they call their new work space in Pittsburgh the “KSHV Laboratory” for the virus that promises to reveal a wealth of information about virology, tumor genesis, and cellular processes.

When Chang and Moore sequenced KSHV's genome in 1996, they were not surprised to find it had many genes typical of herpes viruses. The surprising thing was how many genes were recognizably human. Somehow, KSHV had pirated human genes and taken them as its own. At different times over the course of its evolution, the virus has managed to take copies of human genes from cellular RNA. The process isn't understood, but the results are dramatic.

Humans may have loads of “junk DNA” that seem to have no function, but a virus is the exact opposite. “It's got a small genome,” says Chang. “It's not going to take anything extra that it doesn't need. It just spits out things it doesn't need.”

KSHV isn't the only virus that has pirated human genes, but according to Chang and Moore, none has done so to this extent. In the words of one scientist, KSHV looks like it was “made by a demented tumor biologist,” because if you were going to design something to cause a tumor, these are the genes you would pick. The virus has pirated genes related to cell cycle control, cell proliferation, prevention of apoptosis (programmed cell death), and immune modulation. In other words, it has pilfered the genetic keys to tumor growth and tumor suppression.

“Here's a virus that just laid out all this cell biology and said, ‘Here, study this,’” says Moore. “You know it's so obvious: These are the things that are important for causing a tumor, and there has never been an example of a virus that's like that.”

“Essentially, what you can do is walk down the genome. You can say, I recognize this gene, I know what it does in the human cell, so that means I have an idea of how to study it in the virus.”

Information like this is valuable for much more than understanding KSHV. It's helping scientists learn how all viruses work and understand exactly which cellular mechanisms viruses are targeting. The presence of a pirated gene may even direct scientists to ask further questions about the gene's function in the human cell.

“In essence,” Moore says, “what we and other tumor virologists are working towards is a unified field theory of tumor biology... What we're hoping to do is to be able to say, what are the common features among all tumor viruses? KSHV is very central to that because we can interpret the other viruses in terms of KSHV.”

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Despite its prominence, KSHV is not the only pathogen in Chang and Moore's lab—at least, they hope it isn't. They continue to look for new pathogens with sequence-based methods like RDA.

“There are a number of different lymphomas,” says Moore, “like non-Hodgkin's lymphomas and some Hodgkin's lymphomas, that have a pattern of disease that looks like they might have an infection associated with them. It's not as clear as Kaposi's sarcoma—not nearly

as clear—but there's some evidence for it.”

Chang brings out pictures of a rare cancer of the eye that has emerged in Uganda. The lining of the eye and lids is red and inflamed. In some cases, tumors on the surface of the eye block vision. The disease appears mainly in AIDS patients in a limited geographical area. Just like KS, it could be the result of a virus that doesn't generally reveal its presence except in severely immune-compromised persons.

There are many other diseases, rare and common, that may eventually be traced to an infectious agent. But, Chang notes, “We are likely approaching the end of our ability to identify new pathogens with simple culturing. KSHV is an example where viral fragments were found before the microorganism was cultured.”

To find new pathogens that are difficult or impossible to culture, powerful molecular-based methods are required.

But even for a pair of now-veteran virus hunters, the odds are long, says Chang.

“New pathogen discovery is really high risk,” she says. “And it can be difficult to find a place that will support high-risk research that could take years and years to notch up a discovery.”

“Part of the attraction of Pittsburgh is not only the transplant population, and the pathology banking system that's here, but also just the institutional support for something like this,” continues Chang. “Because we may not find anything. We hope that's not true, but if we do find something it will be because of the support of the University of Pittsburgh. We could have very well stayed at Columbia and kept on working on KSHV; but you know, we started out being virus hunters, I guess. We're very much interested in continuing to find new ways of doing that.”

Those who have worked around Chang and Moore have a lot of faith in their abilities. Richard Wood is a Pitt pharmacology professor and molecular oncologist at Hillman Cancer Center. He went to Westminster College in Salt Lake City, where he first met Moore, then a fellow undergrad. Wood says that the couple has worked so well together for so long that it's hard to separate what it is that each one does differently:

“What you see when you are around them is that they are talking science all the time. It's quite remarkable. So as they pick up their kid from day care and go home, they'll start discussing an experiment. It's just constant dialogue, which I think is a really wonderful resource to have by your side all of the time.” ■

COURTESY CHANG AND MOORE



Chang and Moore at the NIH, after receiving the Charles S. Mott Prize