Profile of Tak Wah Mak

Days after Tak Mak and colleagues (1) revealed a way that cancer cells adapt to environmental stress, the immunologist and his team announced the discovery of a protein that may cause heart failure (2). The two breakthroughs were all in a week's work for Mak, director of The Campbell Family Institute for Breast Cancer Research at the Princess Margaret Hospital in Toronto, Ontario, Canada. Throughout the course of his career, Mak has contributed to over 700 papers, received more than 65,000 citations in leading scientific journals, and garnered numerous prestigious international awards. In 2002, he became a foreign associate of the National Academy of Sciences.

Mak's discoveries have made enormous contributions to researchers' understanding of immunity, particularly as it relates to cancer, arthritis, autoimmune disorders, heart disease, and HIV/AIDS. In 1984, working against all odds, including a rejected research grant, Mak and colleagues (3) identified a cornerstone of modern immunology: the first gene encoding a subunit of the human T-cell receptor (TCR). Mak, who is also a professor in the Departments of Medical Biophysics and Immunology at the University of Toronto, achieved this success, in part, because he challenged conventional scientific thought and forged fresh research paths. Many of his peers have predicted that these qualities will allow Mak, a visionary and prolific researcher, to solve some of the world's most perplexing scientific problems.

Tak Wah Mak.

Unusual Beginning

Mak's journey could never be called conventional. As he says, "The classic profile of a scientist begins with someone at the age of 4 finding a frog in the woods and then becoming in tune with nature. My story was nothing like that."

Born in southern China and raised in Hong Kong, Mak was educated at a religious school deemed among the best in the then-British colony. His high school, directed by Jesuit priests, was extremely well regarded, with parents competing to gain their children entrance. Mak's family urged him to become a doctor, but the intellectual young man had other ideas, and a lot of them. "I was very interested in history, but there were no jobs in history," he says. "I was also interested in math, biology, and chemistry but not so much physics. Basically, I graduated from high school with the goal of avoiding medical school."

During the mid-1960s, Mak's family moved to the United States just as he was about to enter university. "I wanted to go to the University of California, Berkeley, but my mother and her friends said, 'Don't go to Berkeley. It's a hotbed of antiwar activities, and you will never be able to concentrate on your studies,'" he recalls. "I wound up at the University of Wisconsin, Madison, which had one of the top chemistry and biochemistry departments in the nation. Ironically, it also had even more radical antiwar activities than Berkeley."

Mak initially pursued chemical engineering at the University of Wisconsin but soon realized his interests lay elsewhere. He switched his major to biochemistry, earning his bachelor of science degree in 1967 and his master of science degree in biophysics 2 years later. He then moved to Canada and obtained his doctorate from the University of Alberta in Edmonton in 1972.

Three Wise Men

During his studies at the University of Wisconsin, Mak met the first of three men who he says shaped his life's work. Virologist Roland Rueckert was new to the university's biochemistry department and looking for staff. "Roland had a job opening in his lab for a dish washer," Mak says. The job paid $1.25 an hour. After scrubbing test tubes and beakers for a day, Mak inquired if more such work was available. Rueckert replied that there were no more dishes to wash but invited Mak to help with research experiments for the same pay rate. Mak accepted the challenge. "That was the beginning of my scientific career," he says.

At the time, Rueckert was studying the replication and assembly of RNA viruses, particularly picornaviruses. In 1974, Mak coauthored a Rueckert paper published in *Intervirology* (4). The meticulousness of this study, which involved the use of sodium metaperiodate in micromolar concentrations, exemplified Rueckert's approach to science and made a strong impression on Mak. "Everything Roland did was very systematic, thorough, and precise," Mak recalls. "Through Rueckert, I learned discipline and how to organize experiments."

After earning his doctorate, Mak accepted a postdoctoral fellowship at the Ontario Cancer Institute (OCI) in Toronto, where he remains a member of the senior scientific staff. In the mid-1970s, Mak worked in the OCI laboratories of Ernest McCulloch and James Till, the codiscoverers of hematopoietic stem cells (5). "McCulloch was an original thinker and not at all a conventional person," Mak says. "He taught me to challenge dogma and not to believe everything I read." If the young Mak broached a theory, McCulloch would ask for five different speculations based on that single theory. "For me, it was like a new door opened. McCulloch taught me the benefits of combining a careful and methodical approach with thinking freely and beyond the usual boundaries."

In 1980, Mak returned to the University of Wisconsin to learn molecular biology techniques from Howard Temin, who later won the Nobel Prize in Physiology or Medicine for his discovery of reverse transcriptase (6). "That was a very important year for me," Mak says. "I learned to be humble because, after working with McCulloch, I believed I could think freely and make my own hypotheses in an unrestricted way. Howard Temin taught me that my free thoughts also needed to be deep and insightful." Like Mak, Temin was interested in many different disciplines. Temin took great advantage of his extensive knowledge to make unconventional scientific connections. Mak explains that the process is "like turning over a rock in Australia and having it remind you of another rock on a beach in Hong Kong." For Mak, it is a matter of thinking in an

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unconstructed manner while drawing on a wealth of remembered data.

Although Mak also credits his students and postdoctoral fellows with teaching him a great deal over the years, it was these three men—Roland Rueckert, Ernest McCulloch, and Howard Temin—who most strongly influenced Mak during the years preceding his own scientific breakthroughs.

Beating the Odds

After wrapping up his work with Temin, Mak returned to Toronto to establish his own laboratory at OCI. Mak's original team, consisting of postdoctoral fellow Kohei Nagasawa and three other individuals, focused on two separate areas: oncogenic retroviruses and human T-cell leukemia. Their initial goals were to understand precisely how retroviruses can transform cells and to elucidate the mechanisms underlying T-cell differentiation.

At the time, the genes encoding the TCR had proved so difficult to clone that the task was nicknamed "the holy grail of immunology" (7). Laboratories worldwide were pouring millions of research dollars into solving the mystery of how T lymphocytes recognized their targets via a structure that consisted of both an antigen and an MHC molecule. At that time, most researchers believed that the TCR was nothing more than a type of Ig molecule.

Meanwhile, in half of Mak's laboratory, Nagasawa discovered that phorbol esters could trigger "maturation programs" in T-cell leukemias and that the differentiation driven by these programs led to the expression of many new T-cell markers. Concurrent with this work, the other half of Mak's laboratory was performing molecular subtraction experiments to clone genes involved in transformation by oncogenic viruses. Mak used his talent for cross-disciplinary thinking to devise the unique approach of performing experiments on B lymphocytes to isolate T lymphocyte-specific genes. His subtraction method involved screening thousands of clones from a human T-cell leukemia line to determine which corresponded to mRNAs expressed in T cells but not in B cells. Convinced this approach could incidentally also help clone the TCR genes, if indeed they were T cell-specific, Mak quickly drew up a grant proposal. However, the proposal was turned down almost immediately. Mak's team took the letter of rejection to mean that a small Canadian laboratory had no business getting involved in the international, high-powered, big-money race to clone the TCR genes.

Undaunted, Mak and postdoctoral fellow Yusuke Yanagi forged ahead with their time-consuming molecular subtraction work until, on one memorable weekend, the Eureka moment happened. "It was a sunny Sunday afternoon in the summer of 1983," Mak recalls. "I walked into the lab, and there was a stack about 2 ft high of computer sheets comparing the sequences of our T cell-specific genes to everything in the gene bank." About 5,000–6,000 known sequences had been compared at that time. "After scanning through hundreds of pages, I held up one sheet, looked at it from an angle, and there it was—YT35, a clone whose predicted protein sequence was similar, but not too similar, to an Ig's variable, joining, and constant regions. I stared at it for a long time and finally said to myself, 'I can't believe it. This could be the human TCR.'"

Subsequent experiments proved that Mak's suspicion was correct. Also in the summer of 1983, Mark Davis at Stanford University was independently using a related approach to clone a mouse TCR gene (8). The Davis and Mak teams published back-to-back papers in *Nature* in March 1984, announcing their seminal findings. The first line of Mak's abstract understates the tremendous power of the achievement: "We have cloned and sequenced a human mRNA specific for mammalian T lymphoid cells." The discovery skyrocketed Mak to international acclaim and became a defining moment in his career. To this day, many people still consider Mak solely an immunologist, although his research spans many other fields.

Custom-Built "Candy Store"

Mak spent most of the 1980s and the first half of the 1990s studying genes involved in the immune system. In 1988, he joined the growing number of researchers experimenting with genetically modified mice. Mak summarizes, "The basic principle is as follows: If you delete, or knock out, a gene in a mouse embryo's genome and the mouse grows up without a tail, then that gene must be involved in tail formation." To date, Mak and his team have generated about 170 strains of genetically modified mice and defined the functions of most of the proteins encoded by the affected genes. He likens these efforts to "being like a kid in a candy store," because this technique permits exquisite dissection of complex physiological phenomena and leads to a clear and systematic understanding of complex pathways, such as those governing intracellular signal transduction. Mak and his team have used knockout mice to make crucial contributions to immunology, cancer biology, mechanisms of programmed cell death, and immunotherapy. In 1993, Mak's success in these endeavors caught the attention of Amgen, Inc., a leading biotechnology company based in California. That company established a research institute in Toronto where Mak and others could expand their studies of genetic diseases, cancer, and autoimmune disorders. With the generous funding provided by Amgen, Inc., Mak was able to double the size of his team.

Changing Course

Like many others, Mak and colleagues have used genetically modified mice and related approaches to achieve important advances in cancer research. However, Mak has been frustrated by the lack of targeted therapies for this deadly disease. "The FDA has not approved a drug for a new major anticancer target between 2007 and 2010," he points out, even though billions of dollars have been poured into this field, along with the efforts of thousands of scientists.

Popular targets for new anticancer drugs are oncogenes, which are mutated or amplified forms of normal cellular genes. In the 1970s and 1980s, the general scientific belief was that a few oncogenes were the major drivers of most cancers; thus, these genes became the focus of much basic research. In the 1990s and 2000s, emphasis was also placed on identifying tumor suppressor genes (TSGs), which encode proteins that repair mutations and block transformation. Mak's laboratory was heavily involved in this work. Two of the most critical TSGs are p53 and phosphatase and tensin homolog deleted on chromosome ten (PTEN), and Mak's team made major contributions to demonstrating the importance of these genes. Together with Stephen Elledge, Mak and colleagues (9) showed that it is Chk2 kinase that allows p53 to carry out its antitumor functions. Mak's laboratory, along with that of Jack Dixon, was also among the first to show that PTEN negatively regulates the PI3-kinase pathway that promotes the survival of cancer cells. Mak's explorations of the functions of these TSGs eventually led him to investigate genes driving breast tumors. His group's studies of mice lacking the breast cancer susceptibility genes encoding Brca1 and Brca2 showed that these proteins help to control p53 and maintain genomic stability. Mak's work on breast cancer caught the eye of the philanthropic Campbell family in Toronto, who in 2004 tapped Mak to head up a research institute dedicated to finding the causes and a cure for breast cancer.

Mak notes that cancers exhibit hundreds or thousands of different genetic aberrations, with perhaps dozens, if not hundreds, constituting cancer drivers. Moreover, this estimate fails to take into account alleles that may predispose individuals to tumorigenesis or epigenetic

changes, which are alterations to DNA outside genes that affect their expression. "It's overwhelming and disheartening!" he exclaims, considering the challenges. "If you think of a horse and cart analogy, we've been aiming at the horses to stop the carts, but now we are forced to also think about the carts because there are too many different horses." This realization has spurred Mak into radically altering his team's research directions, which he hopes may lead to the emergence of novel targets and new classes of anticancer drugs.

Legacy

The common thread running through Mak's work is a dedication to serving others. He generously shares the reagents and mice generated by his laboratory. No matter what obstacles he faces, Mak believes the straight and narrow path can lead to biological truths. "My lab is committed to understanding the molecular pathways underlying human diseases," he says. "Scourges like cancer and autoimmunity represent an ongoing war, and so we can never give up the fight against them. We can walk away for a while and work on something else, but we must always return to these battles."

Although Mak's work has garnered him several prominent awards, he declines to rest on his laurels and is determined to leave no stone unturned. "I have become acutely aware of what is happening in the clinic," he says. "For years now, clinicians have been teaching scientists as much as scientists have been teaching clinicians. In reality, there may be very few new major biological paradigms left to discover." Mak also says that his greatest joys come from reading published papers or listening to others discuss science and then being able to make unexpected connections with his own work and experiences. "That is what has kept me going," he says. "It becomes a thrill to chase down a new finding in another scientist's work, to link that result to a remembered observation in my own research, and to produce a connection that is new. The more you learn, the more broadly you can relate that knowledge, and the more surprising are the insights you can generate."

Jennifer Viegas, *Freelance Science Writer*

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