MolecularMedicine

A Journey in Science: "Not Lost in Translation"

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Real innovations in medicine and science are historic and singular; the stories behind each occurrence are precious. At *Molecular Medicine* we have established the Anthony Cerami Award in Translational Medicine to document and preserve these histories. The monographs recount the seminal events as told in the voice of the original investigators who provided the crucial early insight. These essays capture the essence of discovery, chronicling the birth of ideas that created new fields of research; and launched trajectories that persisted and ultimately influenced how disease is prevented, diagnosed, and treated. In this volume, the Cerami Award Monograph is by Tak Mak, PhD, Professor, The Campbell Family Institute for Breast Cancer Research, Ontario Cancer Institute, Princess Margaret Cancer Centre in Toronto. A visionary in the field of cancer, this is the story of Dr. Mak's scientific journey.
Online address: http://www.molmed.org
doi: 10.2119/molmed.2016.00176

NO FROGS, NO WAR

My evolution as a translational scientist has been anything but linear. Most researchers first become fascinated with science as youngsters as a result of trapping a tadpole in a pond and watching it grow legs in a jar. Not me. I grew up in Hong Kong and was educated in a leading Jesuit school, with no ponds in sight. I developed a deep love of history in high school and thought about becoming a Catholic priest, but was discouraged by my more practical family. My mother declared that there were no jobs in the history field so I should be a medical doctor instead. My goal became avoiding medical school at all costs, even though I did enjoy biology.

My family moved to the USA in the mid-1960s. While I wanted to attend the University of California at Berkeley, my mother and her friends viewed this institution as a "hotbed of anti-war activities" that would distract me from my studies. To avoid this dire fate, I enrolled at the University of Wisconsin at Madison, famed for its superior chemistry programs. To my mother's chagrin, it soon became even better known for its strident anti-war stance. I nevertheless began my undergraduate major in chemical engineering but became troubled by my classes, which emphasized discipline rather than discovery. After I resisted completing a particularly monotonous assignment, the dean of engineering recommended that I join another faculty. Over the next 4 years, I earned my BSc in biochemistry and MSc in biophysics at the same institution. It was during my time in Wisconsin that I met the first of the three great scientists who would shape my research philosophy.

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Submitted and Accepted for Publication August 4, 2016; Published Online (www.molmed.org) November 1, 2016.

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ROLAND RUECKERT

In the late 1960s, I was a starving student and Roland Rueckert was a new faculty member in the biochemistry department of the University of Wisconsin. I was looking for lunch money and he was looking for lab staff to wash glassware. After I had finished up the dishes, Dr. Rueckert invited me to assist with some experiments, and my introduction to scientific inquiry began. Roland impressed upon me the importance of attention to detail, precision in formulating hypotheses, and objectivity in data evaluation. I learned how to ask and answer scientific questions that were worth pursuing, and how to be bold but meticulous in experimentation. Roland was studying picornaviruses at the time and introduced me to the advantages of studying complex biological phenomena using simple systems. I resolved to emulate Roland and pursue a career in biological science.

ERNEST McCULLOCH

In 1972, I earned my PhD in Edmonton, Alberta, Canada, and then journeyed east for postdoctoral studies at the Ontario Cancer Institute (OCI) in Toronto. My goal was to study oncology and my project was focused on retrovirus genetics and tumorigenicity. In cooperation

with the lab of Alan Bernstein, an OCI investigator affiliated with the leukemia program of Princess Margaret Hospital, I studied an erythroleukemia-associated virus called Friend spleen focus-forming virus (SFFV). In 1978, I used the newly developed technique of molecular subtraction to isolate the RNA sequences of SSFV that could induce an infected cell to undergo malignant transformation (1). This connection with leukemia led me to interact with Dr. Ernest McCulloch and Dr. James Till, the leaders of the leukemia program and discoverers of hematopoietic stem cells. I was intrigued by Dr. McCulloch's contrarian approach to accepted dogma. For every theory I dreamed up, he challenged me to expand it into five new hypotheses, each straying further from conventional wisdom than the previous one. Dr. McCulloch taught me, as is said far too often today, to think outside the box.

HOWARD TEMIN

In 1980, I returned to the University of Wisconsin and joined the lab of Dr. Howard Temin to learn molecular biology techniques. I had grown confident in my ability to think freely, but I soon found I had much to learn from Howard, who had won the Nobel Prize for his elegant discovery of viral reverse transcriptase. This breakthrough not only overturned the central dogma stating that genetic information must flow from DNA to RNA to protein, but also launched the discipline of molecular cloning. I acquired this skill while working with Dr. Irvin Chen to identify the oncogene v-rel, a homologue of NFκB. I also learned to recognize new scientific connections, where the features of a discovery in one field resemble related features in a different field and spark fresh insights. It's a bit like turning over a rock in Australia and having it remind you of another rock in Alaska.

THE T CELL RECEPTOR

I returned to OCI in late 1980 to set up my own lab focused on oncogenic retroviruses and human T cell leukemias. My small but nimble team was the first to clone and sequence my old friend SFFV and identify its oncogene by molecular subtraction. Funding restrictions then pushed me into joining the immunology world's search for the "Holy Grail": the cloning and sequencing of the genes encoding the T cell receptor (TCR). Rolf Zinkernagel and Peter Doherty had discovered that T cells had to recognize both antigen and MHC to become activated. However, the nature of the receptor protein(s) involved was the subject of fierce controversy, with one popular theory holding that the TCR would be composed of immunoglobulin variable sequences spliced onto T cell–specific constant segments. No one predicted that the TCR would actually be specific to T cells. It was serendipity that, on one side of my lab, postdoc Kohei Nagasawa was examining new surface markers on activated T cells, while the other side was performing molecular subtraction assays on oncogenic viruses. With McCulloch and Temin in the back of my brain urging me to think laterally, I decided to identify T cell–specific genes by cloning T cell mRNAs and molecularly subtracting B cell mRNAs. Although granting agencies refused us funding, postdoc Yusuke Yanagi and I compared thousands of cDNAs, looking for sequences expressed by T cells but not B cells. We isolated dozens of T cell–specific sequences, including Lck, CD3 and the mysterious clone YT35. YT35 encoded a protein that was vaguely homologous to the immunoglobulin light chain but contained V-, J- and C-like regions. Imagine our delight when detailed analysis showed that YT35 encoded the human TCR β chain, a finding we published back-to-back in Nature with Mark Davis's cloning of the mouse TCR β chain (2,3). The enormity of our accomplishment was captured by the late Alan Williams in his News and Views commentary "T Cell Receptors: Elusive No More" (4). This breakthrough propelled my lab onto the international scientific scene.

OF MICE AND MORE MICE

It was in 1988 that I capitalized on the results of fateful discussions with my friend Klaus Rajewsky on the value of being able to generate and study genetically modified mice on demand. In the early 1980s, Oliver Smithies, Mario Capecchi and Martin Evans pioneered the technique of homologous recombination in mouse embryonic stem cells. A single gene is mutated or deleted in a mouse blastocyst such that the adult (if the embryo survives development) lacks the feature(s) encoded by that gene. I turned my lab into a cottage factory churning out these knockout mouse mutants and used them to determine the functions of numerous immune response genes, including *Lck*, *CD28*, *CD4*, and *CD8* (5–8). I also became interested in pathways of intracellular signal transduction, those cascades of enzymatic activities that allow a cell to survive and proliferate or trigger its programmed death. In 1993, Klaus Pfeffer in my lab showed that TNFR1 was critical for cell survival because engagement of this receptor activated NFκB. Conveniently, our studies of cell death overlapped our work on T cell responses, since activated T cells die as an immune response ends so as to prevent damage to normal tissues. In 1995, postdoc Paul Waterhouse showed that knockout mice lacking the T cell surface molecule CTLA-4 exhibited rampant lymphoproliferation due to a lack of apoptotic T cell death (9). His paper marked CTLA-4 as a negative regulator of T cell activation and so paved the way toward the receptor blockade method of immunotherapy later developed by James Allison and colleagues (10). By using specific antibody to interfere with CTLA-4 function, one can avoid turning off T cells that have mounted an anti-tumor response, extending their killing of malignant cells. This approach has since been validated by clinical successes in melanoma and lymphoma, and in lung, bladder, kidney and head and neck cancers.

AMGEN DAYS

My lab's dexterity in generating and analyzing knockout mice did not go unnoticed by Big Pharma. In 1993, the California biotechnology company Amgen established the Amgen Research Institute within OCI in Toronto, providing me with huge and very welcome resources to continue my work. We generated scores of interesting mutants, including the CTLA-4–deficient mouse mentioned above, as well as mice lacking ICOS, BCL-10 or MALT1. We turned the power of our knockout mouse factory onto investigating cancer-related genes and learned to use the inducible Cre-loxP system to make conditional mutants in which loss of a gene, or expression of a modified gene, can be turned on or off at a specific time or in a specific tissue. To date, we have created over 200 knockout or genetically modified mouse strains.

Many of our mouse mutants have revealed much about tumor suppressor genes (TSGs). Vuk Stambolic determined that *Pten* is a TSG because it both negatively regulates cytoplasmic signaling promoting cell survival and functions in the nucleus to support DNA repair (11,12). We also generated mutant mice demonstrating the importance of the checkpoint regulator *Chk2* (13), and the DNA damage/repair genes *Brca1* and *Brca2* (14). All was going along swimmingly until 2002, when Amgen dissolved the institute. My group continued to explore the biology of immune responses and cancer cells under the auspices of the Princess Margaret Cancer Centre. In 2004, the late Audrey Campbell of Toronto and her daughters gave us the gift of a lifetime and established the Campbell Family Institute for Breast Cancer Research. This generous new funding allowed us (and other excellent researchers recruited to staff this new institute) to surge forward in our investigations of cancer causes and cures. It also became possible for us to set up a rare academic drug discovery group with the freedom to follow interesting research threads that a conventional biotech/pharmaceutical company might ignore.

HORSES AND CARTS

For me, a major conundrum in cancer research has been the difficulty that we scientists have had in creating new antitumor drugs. The first chemotherapy drugs, which were devised in the 1940s–50s, simply block the rapid proliferation of tumor cells. Unfortunately, these drugs also kill vital normal cells whose vigorous proliferation is essential for good health. The first such agent was nitrogen mustard, identified when doctors in the First World War noticed that the lymph nodes of soldiers and civilians exposed to this chemical warfare agent were reduced in size. In 1942, Louis Goodman and Alfred Gilman of Yale University administered this poison to J.D., a terminal patient with massive growths in his neck. Spectacularly, J.D.'s tumors vanished for a short period before they recurred. However, even with the refinements made to chemotherapy agents over the past 50 years, toxicity linked to their use is still unacceptably high. It remains largely true that, as Moliere commented 350 years ago, "Doctors pour drugs of which they know little, to cure diseases of which they know less, into patients of whom they know nothing."

Oncogenes are altered versions of normal genes, and the idea that blocking oncogene function could cure cancer sparked the "oncogene revolution" in the late 1970s–80s. Consider the analogy of a horse-drawn cart: the cart is the developing cancer and the horses are the oncogenes drawing the malignancy on in its devastating progression. If you shoot the horses, the cart should stop. Accordingly, researchers furiously began to generate "sharp-shooting" drugs to target the protein products of oncogenes, a strategy that has sometimes worked well. For example, the therapeutic monoclonal antibody Herceptin binds to an oncogenic receptor overexpressed on certain subtypes of breast cancers and thwarts their advance (15). However, intensive sequencing of cancer cell genomes has revealed that they vary much more than originally thought, and that it is also the loss of TSG function (rather than a gain of abnormal oncogene function) that drives tumorigenesis. In these cases, shooting the (nonfunctional) horse will have no effect on the runaway cart. In addition, under the selection pressure of drug treatment, cancer cells frequently develop resistance to the applied agent, invoking alternative signaling pathways to circumvent an inhibitor-mediated blockage. Finally, the oncogene scenario does not take epigenetics into account, which is the modification (usually by methylation) of DNA and/or histone proteins that change a gene's expression without altering its DNA sequence. We are only just starting to unravel the nature of tumorigenic changes to the epigenetic control of gene expression, and much work remains to be done. In effect, our cart now has way too many horses pulling it.

THREE WAYS TO UPSET THE CART

The genomes of ~ 20,000 cancer cells have now been sequenced, and although some mutations are shared by a wide range of malignancies, it is clear that it takes much more than a single mutation to drive disease, and that each cancer is unique. This realization has given rise to the concept of "personalized medicine," where one identifies the defect(s) in a patient's cancer cells and then treats the patient with the agents specifically targeting those defects. However, this strategy would require very expensive customized care for each patient, would not guarantee a cure, and does not account for the considerable genetic and epigenetic heterogeneity existing within a single tumor. Administering a combination of anti-oncogene drugs is logical, but rare, because pharmaceutical companies are reluctant to combine their drugs with those of competitors. Moreover, when multiple anti-oncogene drugs have been tried as treatment, patients suffer debilitating side effects. With all this in mind, I have invoked the philosophies of my mentors and have taken to ignoring the horses and focusing on the cart. What common properties do cancer

cells acquire during transformation that distinguish them from normal cells, and how do these properties sustain these bad actors? If we ignore factors driving cancer initiation and instead target "maintenance" properties, can we kill tumor cells without affecting normal cells and thus block cancer progression with minimal toxicity?

Before the discovery of oncogenes, targeting the "cart" was the norm. In 1966, a handful of Nobel Laureates gathered at Lindau to deliberate on "The Prime Cause and Prevention of Cancer." After heated discussions, Otto Warburg declared that cancer is not caused by viruses or rogue genes, saying: "But, even for cancer, there is only one primary cause. Summarized in a few words, the cause of cancer is the replacement of the respiration of oxygen in normal body cells by a fermentation of sugar. Because no cancer cell exists, the respiration of which is intact, it cannot be disputed that cancer could be prevented if the respiration of the body cells would be kept intact." With oncogene targeting reaching its limit, cancer metabolism is again becoming an investigative priority, reviving Warburg's original vision (16).

Scientists worldwide are seeking ways to upend the cancer cart and strip tumor cells of the properties sustaining their growth. Our group is now focused on the metabolic adaptations that allow tumor cells to survive under conditions that kill normal cells. Three such metabolic changes not seen in normal cells are increased energy consumption, elevated levels of biosynthetic compounds, and altered management of reactive oxygen species (ROS). Our studies have implicated: *PARK7* (*DJ-1*), a Parkinson's disease gene that is also an oncogene (17); carnitine palmitoyltransferase-1C, a brain-specific isoform of an enzyme that is involved in β-oxidation and upregulated in cancers to provide redox capacity as well as energy (18); ENTPD5, an ectonucleoside triphosphate diphosphohydrolase that is enhanced in cancer cells to deplete ATP, increase UMP and dampen ER stress (19); and estrogen, which augments the expression in tumor cells of NRF2, the master transcription factor for antioxidation (20,21). Perhaps the best example of altered metabolism in tumor cells is the mutation of isocitrate dehydrogenase (IDH). The altered *IDH* gene generates an enzyme producing an abnormal metabolite, D2HG, which is oncogenic (22,23). Recent clinical trials of inhibitors of mutant IDH enzymes have had very exciting initial results, reducing D2HG levels in leukemia patients and decreasing numbers of cancerous blood cells.

A second cart-disrupting strategy in my lab targets the genomic instability of advanced tumor cells. Cancer cells forced to adapt to a high level of oxidative stress incur damage to their DNA and DNA repair systems, making them genomically unstable and often aneuploid (containing an abnormal number of chromosomes). Although this anomaly should make it hard for the tumor cell to divide, the cell can deregulate specific genes allowing mitosis to proceed regardless. Critically, these genes are less important in genomically stable tumor cells and dividing normal cells. We have identified 2 such genes, encoding the mitotic kinases PLK4 and TTK (24–26). Both of these enzymes are involved in centriole duplication and maintaining genomic stability, and TTK also participates in the spindle assembly checkpoint. Our academic drug discovery group has generated specific, potent and fairly nontoxic inhibitors for these enzymes, and we have treated human cancer cell lines and mice bearing xenografts of human cancers with these drugs. To our elation, we have seen markedly reduced tumor cell growth. Clinical trials of these agents in human cancer patients are now under way. Ironically, this approach works because our drugs increase the level of genomic instability to a point where even tumor cells can no longer cope and are killed. It is our hope that these agents are just the first of a new class of drugs that specifically target the cart, leaving the horses with nothing to pull.

Our last strategy to overturn the cart brings us back full circle to immunology, since there is no body system with greater powers of discrimination between normal and diseased cells than the immune system. Tumor cells often express abnormal surface proteins that can be recognized by T cells and frequently provoke localized inflammation drawing immune cells to the area. As noted above, blocking the action of the negative regulators CTLA-4 and PD-1 sustains T cell activation and has provided considerable clinical benefits. Much effort is now being expended to identify molecular interventions that can be combined with blockade agents. However, we still do not completely understand how leukocyte migration is controlled, how to make "visible" tumors that do not express abnormal surface proteins (and so are ignored by T cells), and how to get around the evasion mechanisms and secreted molecules that cancers use to shut down attacking leukocytes. All these issues are under intensive investigation by my lab and many others, with new progress being reported every day.

CLOSING THOUGHTS

I have been fortunate in my career thus far, benefitting greatly from the advice of great mentors and the support of generous funders. I have been lucky enough to lead teams of extraordinary young researchers and superior technicians who are able and willing to go the extra mile to generate groundbreaking data. To see concrete results emerging from cross-disciplinary thinking, and to see the thrill on a young scientist's face when she or he makes a truly new and insightful connection, brings me great joy. I truly believe that sharing data widely and building continuously on the work of all will eventually allow us to defeat disorders like autoimmunity and cancer.

Most historians and journalists traditionally brand any researcher associated with a scientific breakthrough as brilliant, while labeling those with less spectacular results as unsuccessful.

After four decades of scientific investigation, I feel qualified to challenge this simple stereotype. At least 50% of success in any lab is luck and being in the right place at the right time. Technologies are also enormously important in scientific breakthroughs. While few scientists are or should be testing old hypotheses with old technologies, investigating new hypotheses with old technologies can also be challenging. As pointed out by Richard Feynman, "If you are getting nowhere with an old technology, having a fast technology only gets you nowhere faster!" Similarly, testing new theories with innovative technologies can be intellectually demanding and downright puzzling if all the bugs have yet to be worked out, or if the technology generates huge amounts of "big data" that swamp the mind. I feel comfortable saying that most of my lab's success has come from testing old hypotheses with new technologies such as subtractive hybridization and genetically modified mice. Indeed, some of our most noteworthy observations seem to have come from testing no hypothesis with new technology. Nonetheless, I have found that the most important ingredient in scientific discovery is the recognition of human factors: who will work best on which project, who will change tack with the tide, and who will swim against the tide and fashion new approaches. As I coast toward the sunset of my career, I have come to realize that, while 20th-century science was generally driven by basic discoveries and paradigms, 21st-century research will be dominated by clinical intuition. My future counterparts will have to insightfully dissect basic research to seize upon those findings that can be readily translated to the clinical setting to help patients. Thoughts and trends change, approaches to treat diseases evolve, and science digs deeper step-by-step to find new therapeutics that can reduce human suffering. I look forward to learning about many such translational delights in the coming years.

ACKNOWLEDGEMENTS

Many thanks are due to all former and present postdoctoral fellows, undergraduate and graduate students, and technicians in my lab. I am also grateful to the Canadian Institutes of Health Research and many other funding agencies for supporting my lab's work over the years. Finally, I wish to acknowledge the unfailing assistance of our administrative team, headed by Irene Ng.

REFERENCES

1. Mak TW, Penrose D, Gamble C, Bernstein A. (1978) The Friend spleen focus-forming virus (SFFV) genome: fractionation and analysis of SFFV and helper virus-related sequences. *Virology.* 87:73–80.
2. Chien Y, *et al.* (1984) A third type of murine T-cell receptor gene. *Nature.* 312:31–5.
3. Yanagi Y, *et al.* (1984) A human T cell–specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains. *Nature.* 308:145–9.
4. Williams AF. (1984) The T-lymphocyte antigen receptor—elusive no more. *Nature.* 308:108–9.
5. Fung-Leung WP, *et al.* (1991) CD8 is needed for development of cytotoxic T cells but not helper T cells. *Cell.* 65:443–9.
6. Molina TJ, *et al.* (1992) Profound block in thymocyte development in mice lacking p56lck. *Nature.* 357:161–4.
7. Rahemtulla A, *et al.* (1991) Normal development and function of CD8+ cells but markedly decreased helper cell activity in mice lacking CD4. *Nature.* 353:180–4.
8. Shahinian A, *et al.* (1993) Differential T cell costimulatory requirements in CD28-deficient mice. *Science.* 261:609–12.
9. Waterhouse P, *et al.* (1995) Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. *Science.* 270:985–8.
10. Leach DR, Krummel MF, Allison JP. (1996) Enhancement of antitumor immunity by CTLA-4 blockade. *Science.* 271:1734–6.
11. Stambolic V, *et al.* (1998) Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. *Cell.* 95:29–39.
12. Bassi C, *et al.* (2013) Nuclear PTEN controls DNA repair and sensitivity to genotoxic stress. *Science.* 341:395–9.
13. Hirao A, *et al.* (2000) DNA damage-induced activation of p53 by the checkpoint kinase Chk2. *Science.* 287:1824–7.
14. Hakem R, *et al.* (1996) The tumor suppressor gene *Brca1* is required for embryonic cellular proliferation in the mouse. *Cell.* 85:1009–23.
15. Pegram MD, *et al.* (1998) Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. *J Clin Oncol.* 16:2659–71.
16. Warburg O, Posener K, Negelein E. (1924) Über den Stoffwechsel der Tumoren. *Biochemische Zeitschrift.* 152:309–44.
17. Kim RH, *et al.* (2005) DJ-1, a novel regulator of the tumor suppressor PTEN. *Cancer Cell.* 7:263–73.
18. Zaugg K, *et al.* (2011) Carnitine palmitoyltransferase 1C promotes cell survival and tumor growth under conditions of metabolic stress. *Genes Dev.* 25:1041–51.
19. Fang M, *et al.* (2010) The ER UDPase ENTPD5 promotes protein N-glycosylation, the Warburg effect, and proliferation in the PTEN pathway. *Cell.* 143:711–24.
20. Gorrini C, *et al.* (2013) BRCA1 interacts with Nrf2 to regulate antioxidant signaling and cell survival. *J Exp Med.* 210:1529–44.
21. Gorrini C, *et al.* (2014) Estrogen controls the survival of BRCA1-deficient cells via a PI3K-NRF2-regulated pathway. *Proc Natl Acad Sci U.S.A.* 111(12):4472–7
22. Cairns RA, Mak TW. (2013) Oncogenic isocitrate dehydrogenase mutations: mechanisms, models, and clinical opportunities. *Cancer Discov.* 3:730–41.
23. Dang L, *et al.* (2009) Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature.* 462:739–44.
24. Laufer R, *et al.* (2013) The discovery of PLK4 inhibitors: (E)-3-((1H-Indazol-6-yl)methylene) indolin-2-ones as novel antiproliferative agents. *J Med Chem.* 56:6069–87.
25. Laufer R, *et al.* (2014) Discovery of inhibitors of the mitotic kinase TTK based on N-(3-(3-sulfamoylphenyl)-1H-indazol-5-yl)-acetamides and carboxamides. *Bioorg Med Chem Lett.* 22:4968–97.
26. Mason JM, *et al.* (2014) Functional Characterization of CFI-400945, a Polo-like Kinase 4 Inhibitor, as a Potential Anticancer Agent. *Cancer Cell.* 26:163–76.

Cite this article as: Mak T. (2016) A journey in science: "Not lost in translation". *Mol. Med.* 22:675–9.