In the Battle Against Cancer, Sequencing is a Powerful Weapon

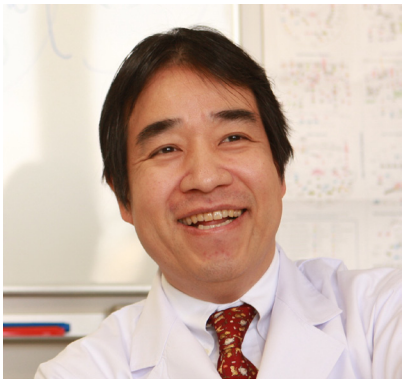
How one professor is adapting cutting-edge science to identify new therapeutic targets for cancer patients

Introduction

Nearly 20 years ago, Dr. Hiroyuki Mano took on his first patient, a man with acute myeloid leukemia and a very dim prognosis. In those days chemotherapy was still quite naive, often administered like a bullet shot into the dark, aimed at a destructive yet largely invisible target. After six months of intensive chemotherapy, the patient succumbed to the fight, but not with the cancer—it was the onslaught of drugs that eventually broke his body's threshold for survival. In the end, a fungal infection, brought on by diminished immune protection as a result of the drugs, is what overcame him. Shaken by the experience, Dr. Mano believed that there had to be a better, more logical, more sophisticated approach to target disease pathways. This mind-set ultimately shaped his career, eventually leading him to use high-throughput sequencing as a tool to provide better treatment for patients. He decided to return to academia to earn a Ph.D. and started a medical genomics research lab focused on understanding the genetic mechanisms behind malignant transformation. Today, at The University of Tokyo and Jichi Medical University in Japan, Dr. Mano is achieving great success in this area, discovering and characterizing a critical oncogene implicated in certain lung cancers and developing innovative approaches to screen for disease-causing mutations.

Dr. Mano began his research career studying acute myeloid leukemia, a logical choice given his hematology background and experience with leukemia patients. This work led to the discovery of many important genes, including a key TEC tyrosine kinase essential to immunological development. While his work was academically good science that resulted in numerous publications, he felt unsatisfied, his true aspiration still unmet. He had started down the research path to find better ways to help patients, yet his work thus far had not furthered that objective. He recalls, "My discoveries had not helped to cure any patients, nor did they provide early detection of a cancer. So when I became a professor, I dedicated the rest of my scientific life to translational medicine, focusing on research that would directly help the lives of patients."

Pondering this idea for a while, he realized that in order to develop better treatments, the scientific community needed better ways to identify essential growth drivers for the disease. There really wasn't an effective general method to screen for the gene mutations that promoted the indispensable growth seen in cancer. To address this need, Dr. Mano developed a highly sensitive functional screening system based on retroviral libraries created from small amounts of cDNAs isolated from a clinical cancer specimen. Using a basic contact-inhibition assay with fibroblasts, he could identify which virus particles harbored the growth-promoting mutations. Identified particles carrying the genetic mutation were used to create transgenic mice so that the phenotypic result could be observed. In principle, the system could be employed to screen for mutations associated with any type of cancer.



Hiroyuki Mano, MD, Ph.D., Professor at the University of Tokyo and Jichi Medical University.

A Major Breakthrough for Lung Cancer Research

Using his functional screening assay, Dr. Mano made a key discovery for lung cancer research, identifying a critical onco fusion gene, EML4 (echinoderm microtubule-associated protein-like 4)—ALK (anaplastic lymphoma kinase). Generated by a small inversion within the short arm of human chromosome2, EML4-ALK encoded protein kinase is found in 4–5% of non-small cell lung cancers. When EML4-ALK was expressed in lung epithelial cells in transgenic mice, they manifested hundreds of lung cancer nodules very early in development. Dr. Mano explains, "Usually an oncogene transgenic model system will show cancer 3–6 months after birth, but this oncogene was so potent that the mice already had hundreds of lung cancer nodules when they were born." These observations suggested that the identified onco fusion gene was the essential growth driver of the disease.

Fusion genes are gene hybrids that can occur from the translocation, deletion, or inversion of a chromosomal segment that places two previously separated genes within very close proximity to each other. Sometimes, the resulting hybrid gene will encode products that exhibit very different activity than either of the two genes individually. Fusion genes are implicated in a number of different cancers, including hematological cancers, sarcomas, and prostate cancer. In the case of EML4-ALK, the gene product is a fusion-type protein tyrosine kinase.

To assess the viability of the EML4-ALK encoded kinase as a potential drug target, Dr. Mano treated his mice with a known kinase inhibitor. When he did, the tumors were rapidly eradicated from the lungs, suggesting that ALK was a promising therapeutic target. Naturally, a number of pharmaceutical companies have now begun developing compounds targeting this kinase activity, some of which have shown great early results in clinical studies.

Identifying Novel ALK Mutations through Sequencing

Ongoing clinical trials with tyrosine kinase inhibitors, such as crizotinib, have shown pronounced therapeutic activity in cancer patients with the EML4-ALK variant. Unfortunately, in some cases, the tumors have not responded to the treatment, or became resistant after an initial response. To obtain a more detailed analysis of the genetic mechanisms underlying these occurrences, Dr. Mano turned to high-throughput sequencing technology. This approach has allowed him to uncover important new insights about the disease.

In a recent case study published in the New England Journal of Medicine, Dr. Mano's lab used deep sequencing to evaluate a lung cancer patient whose EML4-ALK–positive tumor developed resistance to crizotinib. The patient's symptoms initially improved with the crizotinib treatment, but after five months, the tumor abruptly started to grow again. Dr. Mano speculated that it might have acquired secondary genetic mutations that conferred resistance to the drug. To investigate this possibility, he sequenced cDNA from the tumor specimen using the Illumina Genome Analyzer$_{IIx}$. Sequencing data revealed two previously uncharacterized mutations that had developed in some cells within the tumor. Follow-up analysis demonstrated that these independently occurring mutations were indeed capable of conferring resistance to the treatment. Computational modeling of the protein showed that the mutations likely caused key structural changes that modulate the kinase inhibition. Understanding the mechanisms behind this drug resistance may lead to new therapies for patients with tumors harboring EML4-ALK that have these acquired mutations.

A Sequencing-Based Cancer Screening System

Having great success with EML4-ALK in non-small cell lung cancer, Dr. Mano wanted to devise a more general method for identifying oncogenes in any type of cancer, this time using next-generation sequencing. His primary considerations for the sequencing-based system were that it had to be feasible for any small lab to run, and in addition to point mutations and indels, it had to be able to detect fusion genes, his specialty. Traditional exome capturing systems are well capable of detecting point mutations and indels within genes, but the probes from these systems are incapable of capturing fusion genes. Exon-capture purification of genomic DNA eliminates information about the introns, where gene fusions mostly occur. For this reason, Dr. Mano decided to develop custom RNA probes to capture cDNAs, since some of these should cover the ligation points in gene fusions. From the pull-down sequences, he could screen the 5' and 3' ends of the reads against the RefSeq database to see if they align to the same gene or if they align to two separate genes, which would indicate occurrence of a gene fusion. His lab is now has an effective method for identifying the disease-causing aberrations. Of their ongoing research, he says, "By doing some other pipeline, we can now identify our gene fusions. So currently we are doing very extensive screening for various human cancers using this cDNA capturing sequencing system."

Dr. Mano's sequencing-based cancer screening system also allows him to generate very high sequencing read depths, which are critical for detecting variants that occur at low frequency within the tumor cell population. As he explains, "Conventional genomic sequencing works well for variant detection in samples where nearly all the cells in the specimen contain the mutation. But for mixed-cell tumors like in breast cancer or prostate cancer, only a small percentage of the cells in specimen are cancer cells." In these cases, standard sequencing coverage depths of 30–40× are not sufficient to detect the mutations occurring at such low frequency within the cell population. However, with his cDNA capture system, he can achieve read depths up to 10,000×, and sometimes more, depending upon the gene expression level. He elaborates, "For intermediately expressed genes, 1,000× coverage will generally provided the sensitivity needed to detect the gene mutation within a small proportion of cells."

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For Dr. Mano, deep sequencing is transforming his ability to divulge the genetic underpinnings that drive the progression of cancer. As he describes his experience, "Illumina sequencing is like pencil or a personal computer in this era—you can't be without it. It has been a tremendous help to us for this type of work. We have been able to run a huge number of sequencing projects within a reasonable time and budget" With the perpetual goal of developing better approaches for molecular target therapy, Dr. Mano has continually adapted cutting-edge science to address the most pressing needs for cancer patients. Using deep sequencing, he is developing powerful new methods to identify cancer-causing variants, diagnose patients, and, ultimately, help pave a path to better disease treatments.

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