Unraveling the Prostate Genome

By Charlie Schmidt

It used to be that scientists looking for the genetic roots of cancer could sequence only one DNA molecule at a time. Today, "massively parallel" techniques allow scientists to sequence billions of DNA molecules at once. That's led to a sharp drop in analytical costs and to the ability to sequence entire cancer genomes. In this new wave of genetic research, perhaps 100 cancer genome sequences are in various stages of completion. Scientists who study them will be able to see all the genetic variation in a given cancer cell—the whole spectrum of changes and rearrangements that might unleash tumor growth—not just simple spelling errors or deletions in DNA.

Last February, researchers from the Broad and Dana–Farber cancer institutes, in Boston, and Weill Cornell Medical College, in New York City, hit a milestone by publishing the entire prostate cancer genome in *Nature*. Whereas most published studies so far have presented just one cancer genome, these researchers sequenced seven, along with matched control genomes from noncancerous cells taken from the same patients. Each patient harbored tumors of at least T2 staging and Gleason grade 7 scores or higher.

Ultan McDermott, Ph.D., a group leader in the cancer genome project at the Wellcome Trust Sanger Institute in Cambridge, UK, says the *Nature* report represents a new trend toward sequencing more and more tumors of the same cancer type—an effort that will over time allow scientists to find rare but biologically significant mutations occurring in small percentages of patients. "We need that list of mutations before we can start looking for new drug targets," said McDermott. "That's going to be the second chapter in this research, but we can't get there until all the driver mutations—the ones that really set cancer in motion—are identified."

McDermott emphasized that the prostate cancer genome had deep coverage, meaning it had been sequenced repeatedly to ensure accuracy. What it didn't reveal, said Howard Soule, Ph.D., chief science officer at the Prostate Cancer Foundation in Santa Monica, Calif., are defining, druggable mutations amenable to current therapy, in the way that trastuzumab can treat Her2/neu mutations in breast cancer, for instance. Instead, the genome revealed a complex landscape littered with all types of variations, including a remarkably large number of gene fusions, or rearrangements–meaning chunks of DNA that were broken off from their normal place in the chromosome and reattached somewhere else. "You wonder how any cell can harbor all those translocations and survive," Soule said. "That's what we need to figure out."

Role of Gene Fusions

Going into the study, scientists already knew that about half of all prostate cancer patients harbor a gene fusion that links TMPRSS2 (an androgen-sensitive protein) to ERG (an oncogene). Arul Chinnaiyan, a professor of pathology and urology at the University of Michigan Health System in Ann Arbor, discovered the TMPRSS2–ERG fusion in 2005. Before that discovery, scientists had seen only gene fusion–based blood cancers, Chinnaiyan said. Since then, roughly 25 more fusions have been detected in prostate cancer, although TMPRSS2–ERG remains the most common.

Berger's research team split the seven tumors selected for the study into two groups: three having the fusion and four without it. "We wanted to see whether there were big differences between the two groups that might lead to the discovery of new genes," Berger said.

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The results revealed differences in genomic expression between the two groups. Specifically, among tumors with the TMPRSS2–ETS fusion, genetic breakpoints—the chromosomal locations from which genes break free to create fusions somewhere else—were all located in areas where protein transcription is active, whereas breakpoints in the non-TMPRSS2 group were concentrated in regions where it wasn't. Although the clinical significance of that difference has yet to be determined, scientists couldn't have detected the difference had they limited themselves to protein-coding regions, called exons, instead of sequencing the whole genome, Berger said.

Another key difference, he said, is that breakpoints in the TMPRSS2-positive genomes disrupted two other genes involved in cancer growth: PTEN, a tumor suppressor, and its helpmate, MAG12. According to Berger, that observation clarified a known correlation. "We knew from the literature that PTEN and TMPRSS2 interact at a basic level and that each participates in tumorigenesis," Berger said. "What's interesting is that every sample where we found this TMPRSS2 fusion, we also found PTEN disruption.

Berger's new study revealed persistent, complex rearrangements in a newly discovered tumor suppressor called CADM2, but only in the TMPRSS2-negative group. "You can think of those tumor suppressor rearrangements as collaborating events in prostate cancer that occur in addition to gene fusion," Chinnaiyan said. "Broadly speaking, if we can begin to understand specifically which fusions and other molecular events account for a patient's prostate cancer, we might be able to prescribe drugs that attack those pathways in a rational, personalized way."

So then why don't scientists target TMPRSS2–ETS, a driving mutation present in half of all diagnosed cases? Because transcription factors in general don't make easy drug targets, Chinnaiyan said. "It's tough to disrupt DNA–protein binding, which is characteristic of transcription factors. "So we're trying to block ETS indirectly by going after downstream targets." Meanwhile, he said, scientists might achieve faster results by going after kinase fusions, such as those involving SLC4A3—an androgen-regulated solute carrier protein—and the protein kinase BRAF.

According to Chinnaiyan, these mutations appear to be mutually exclusive: Patients who harbor the ETS fusion don't express the BRAF fusion and vice versa. And that suggests patients can be subclassified into genomic categories matched to different outcomes, he said. BRAF mutations, for instance, which show up in about 1%–2% of prostate cancers, seem to be especially aggressive. "What's exciting is that if BRAF fusions are driving mutations in these cases, they might also be amenable to therapy," Chinnaiyan said. "We've already got kinase inhibitors that we might be able to use against this rare subset of prostate cancer. One example is sorafenib, which is already approved, and other kinase inhibitors being tested now might be available for use in prostate cancer within a few years."

Confronting Complexity

Vasan Yegnasubramanian, M.D., Ph.D., at the Johns Hopkins School of Medicine, agreed that genomic subclassifications in prostate cancer are a step in the right direction. But he added that the rarity of the BRAF mutation merely underscores prostate cancer's complexity. To resolve one of the biggest questions about the illness—which cancers are likely to progress and which aren't—scientists need to identify more rare mutations, he said. "And as we add them all up, we'll begin to come up with an answer," he said. That's a crucial need, Chinnaiyan said, because most prostate cancers are indolent. Only 20%–30% become aggressive and metastatic, and since distinguishing among them is challenging, many patients get unnecessary treatment at a great societal cost.


Howard Soule, Ph.D.

Predictive mutations might well emerge from the work of the International Cancer Genome Consortium (ICGC), an organization with 20 member nations aiming to sequence 50 cancer types 500 times, for a total of 25,000 tumor genomes. McDermott said that with such a robust research protocol, it's likely that most rare mutations affecting at most 5% of all cancer patients will be identified. Most of the rare mutations identified so far, he said, including the BRAF mutation in prostate cancer, were revealed by hypothesis-driven research that homed in and confirmed the role of a suspected target. With the ICGC's approach, however, new mutations will be uncovered without any a priori assumptions about their role in cancer.

McDermott said the last decade, he said, has been less about medical advances from genomics than about basic science and technical progress leading to better analytical tools. "So now we're standing on the shoulders of giants that will make the promise of the human genome project possible," he said. Much of the activity to come, he said, will focus in particular on "exomes," or genomic stretches that consist only of protein-coding DNA. According to McDermott, these regions account for just 1%–2% of the entire genome, so they give limited, though important, information. Yegnasubramanian points out that although exomes offer more in their analytical yield, whole-genome sequencing reveals more about structural rearrangements that—as the

prostate cancer genome reflects—can often involve noncoding genomic portions of the chromosome.

Reflecting on the prostate cancer genome, Colin Collins, Ph.D., professor of urologic sciences at the University of British Columbia, acknowledged that scientists confronted with its bewildering array of rearrangements, deletions, amplifications, fusions, and other rare genomic events might wonder whether they're looking at biological noise. But what's more likely is that the disease's complexity serves a biological purpose, said Collins, who sits on the Canadian Prostate Cancer ICGC steering committee. In Collins' view, rare events in prostate and other cancers will probably coalesce into a set of defined pathways that might be vulnerable to drug therapy.

"You have to remember that nature evolves complexity for a reason," he said. "To me, that's the real message of this *Nature* paper: that prostate cancer has this great complexity, and this shouldn't come as a surprise when you consider that tumors have to grow, metastasize, escape immune surveillance, recruit a blood supply, and do all those things that they're not supposed to do. … It's only by understanding that complexity that we're going to make progress on this disease."

 DOI: 10.1093/jnci/djr167