RESEARCH PROFILE

# Michael Wigler

## A game-changing technology in cancer

In recent months, Professor Michael Wigler has been writing and speaking publicly about what may be the Next Big Thing in biology. Called single-cell analysis, it's a new way of learning about what's going on inside cells. When it is more fully developed in the years just ahead, it will provide a clearer view than we've ever had of how cells work and what goes wrong when they're not working right — for instance, in cells of a cancerous tumor or a diseased heart or a deeply depressed brain.

Wigler, a geneticist who is the American Cancer Society Research Professor at Cold Spring Harbor Laboratory, considers single-cell analysis "an amazing, transformative technology — a total game changer." He thinks it is very likely to bring near-term benefits for human health, at first in the area of diagnostics, and over time in the way serious illnesses are treated. We focus in this article on cancer.

"Single-cell analysis" actually refers to several emerging technologies and methods. What they have in common is their object: living cells, considered one at a time. Looking in various ways at 100 to 1000 cells sifted from a single sample of tissue, blood or urine will provide a snapshot of what genes are being expressed at a moment in time, or what proteins are present in the cell's cytoplasm.

These things are possible to determine today at comparatively low resolution, using painstaking and costly methods. In addition to getting much more detailed results, what's new about single-cell analysis is being able to consider the varied properties of a single cell — which are in flux over time, in ways we don't yet understand — in the context of knowing that cell's full genome sequence. Single-cell genomes have not been technically possible to ascertain until now. [see sidebar: "Single-cell analysis: in brief"]

The entire package of single-cell data will enable us to understand in unprecedented ways how cells differ subtly from one another. It's information that opens new windows on the biology of normal cells as well as on human pathology.

Techniques usually employed in research and commercial biomedical testing yield results that represent average readings of the properties of *millions* of cells in a given sample. You can learn important things from averages. But as Mike Wigler observes, processes pertinent to illness "are often happening in *rare* cells within a large population. So when you analyze a whole population at once — say, the mass of cells removed in a tumor biopsy — you miss these things."

Wigler expects that single-cell techniques will transform cancer diagnosis and treatment, making it possible not only to detect cancer cells much earlier, in some cases even before a detectable tumor forms, but also to know how best to treat tumors that have formed and accurately predict how they will respond to therapies.

## An opinion to be taken seriously

"One of my dreams," Wigler says, "is that any of us will be able to walk into a doctor's office, he'll be able to draw blood, and there'll be a fairly routine and inexpensive test that tells you within a few hours if you have cancer somewhere, and where in your body it is."

This dream should not take long in becoming reality, Wigler predicts. The technologies for such a test are now being developed at CSHL and elsewhere and should be available within 2 to 5 years. While this is only an estimate, Wigler's is an opinion to be taken seriously. He is a scientist of remarkable and diverse accomplishment, a consistent innovator whose deep thinking on big problems and long list of seminal insights has earned him the respect of his peers and a reputation for seeing things that other people fail to perceive. One recent example is his 2007 "unified theory" of autism's genetic causation, which surprised many by predicting an important role for spontaneously occurring, non-inherited mutations. This and other aspects of the theory are so far being confirmed in research at CSHL and other institutions.

A math major at Princeton who after graduation began training at Rutgers and later Columbia to be an M.D., Wigler was recognized by his mentors to have a gift for abstract thinking. Leaving his medical studies behind, he found his niche while earning a Ph.D. in microbiology at Columbia in the mid-1970s in the lab of Dr. I. Bernard Weinstein.

Wigler's first big ideas, incubated in the Weinstein lab, were whoppers: a pioneering method (called transfection) of transferring DNA between animal cells; and a method called co-amplification that involves getting one gene to associate with another, making it possible to mark them for subsequent selection. Completed together with Richard Axel and Saul Silverstein, the latter method, whose potential in drug development Axel appreciated, famously earned Wigler's alma mater Columbia a billion dollars in patent revenues, and instant respect for the young microbiologist.

When he joined the CSHL faculty in 1978, Wigler was focused on using the techniques he had developed at Columbia to study cancer. His group was first to isolate a mutant gene from a human cancer that when placed in a "normal" cell could cause that cell to undergo cancerous transformation. The mutant gene was *H-ras*, and its co-discovery in 1981 by Wigler and an independent team at MIT helped usher in a historic period of discovery in cancer genetics.

Wigler's discovery and others after it were dependent on the tools he developed at Columbia. It marked the beginning of a career pattern. To this day, Wigler thinks of himself as a tool-builder.

## Isolating 'signal' from 'noise'

In the 1990s as the age of genome sequencing dawned, Wigler and colleagues, including Nikolai Lisitsyn and Rob Lucito, invented tools with which to compare two genomes. The first version of this idea, called representational difference analysis or RDA, was the answer to a problem that cancer geneticists wanted to solve but couldn't, for lack of a tool. Precious insights awaited if one could reliably compare, for instance, the genome in cells sampled from a patient's breast cancer with genomes in that patient's healthy tissue. How, precisely, did the "cancer" genome differ?

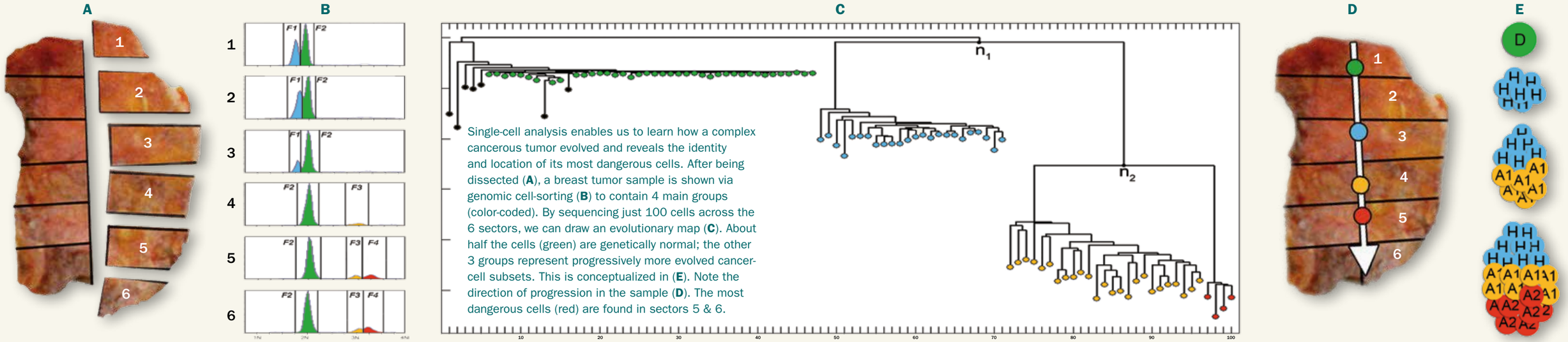
"I used to call this the fundamental problem of biology," Wigler says, referring to the problem of genomic comparison. It involves a challenge that recurs throughout his work: how to isolate a meaningful signal that is

### Single-cell analysis: in brief

We have been sequencing whole genomes for over a decade, but not until recently has it been possible to think of getting a full genome sequence from the DNA contained in a single human cell. Current methods piece together a single genome by assembling, roughly, a billion bits of DNA derived from a million cells. The resulting genome therefore represents a "consensus version" of the DNA sequences found across the entire population of cells that contributed to the assembly.

But what if you wanted to know how the genome of a single cell — say, a cancer cell in a particular part of a prostate tumor — compared with another cell in the tumor? Or in a metastatic outpost of the primary cancer? It was not possible to make such a comparison of single cells until the Wigler lab figured out how to capture enough of a genome from the DNA in one cancer cell to read copy number variations and thus get a meaningful picture of the mutations in that cell. In refinements of this approach, Wigler's team has classified different clonal subpopulations of cancer cells in tumors and is now making the procedures much more cost-effective — a condition for clinical utility.

Single-cell analysis, whether in cancer or in other applications, brings other technologies into play, involving, for instance, the precise measurement of RNA messages in the nucleus at a given moment in time — an index of what genes are being expressed; or fine-grained accounting of the many types of proteins present in the cytoplasm of a single cell. It is really as an ensemble of technologies that single-cell analysis becomes extremely powerful.




Single-cell analysis enables us to learn how a complex cancerous tumor evolved and reveals the identity and location of its most dangerous cells. After being dissected (**A**), a breast tumor sample is shown via genomic cell-sorting (**B**) to contain 4 main groups (color-coded). By sequencing just 100 cells across the 6 sectors, we can draw an evolutionary map (**C**). About half the cells (green) are genetically normal; the other 3 groups represent progressively more evolved cancer-cell subsets. This is conceptualized in (**E**). Note the direction of progression in the sample (**D**). The most dangerous cells (red) are found in sectors 5 & 6.

embedded in an ocean of distracting noise. In considering two highly complex and nearly identical objects — like two genomes — how could one isolate what's different about them, while setting aside all the things about them that aren't important in the context of answering a specific question?

RDA and a related technology Wigler's team later developed called ROMA, which greatly increased RDA's power by adapting it to microarray technology, made such comparisons possible. This major advance and others facilitated studies that greatly changed our picture of many diseases, including cancer. We learned that cancer is not a single disease but many, with a bewildering array of genomic signatures.

## Analyzing heterogeneity within tumors

"Cancer genomes have lots of mutations, and in the past we have explored these by extracting DNA, comparing it to normal DNA of the person, and from that getting an 'inventory' of [genetic] problems," says Wigler. "As far back as 2002, though, I've had the idea of trying to find out about cancer by examining the genome in *single cells* from a tumor."

If there were no rhyme or reason to tumors — if they multiplied chaotically, and utterly unpredictably, as many once believed — the advance represented by single-cell analysis would not be so useful in cancer. "There was no reason to assume that cancers were genetically heterogeneous; but the advance represented by single-cell sequencing analysis showed otherwise," explains Wigler.

His team has been the first to figure out how to determine gene copy number in individual human cancer cells, a critical first step in getting a useful genome readout from single cells. There's an important backstory here: in the early part of the 2000s, Wigler and colleagues made a landmark discovery: We all harbor gene copy number variations (CNVs), meaning that instead of the two versions of each gene that we're presumed to possess (one copy inherited from each parent), the average person has several dozen spots along their chromosomes where there is either too much or too little DNA, relative to the "reference" human genome. Most CNVs are innocuous.

In cancer, Wigler and others have observed recurring chromosome "breakpoints" marking places where small or large segments of DNA are either amplified or missing. These are good places in the genome to look for oncogenes and tumor-suppressor genes. In cancer cells one might see many extra copies of an oncogene like *K-ras* or Myc, or the deletion of one or both copies of a critical tumor suppressor gene like *PTEN*.

"Thanks to the bravery of a postdoctoral student in our lab, Nicholas Navin," says Wigler, and the collaboration of Wigler's longtime research colleague Jim Hicks, a CSHL Research Professor, his lab has devised a protocol for sifting massive numbers of cells from a tissue sample to find a much smaller number likely to bear the genomic marks of cancer — and to then sequence their genomes, cell by cell, using high-throughput technologies.

In 2009, Navin (now on the faculty of M.D. Anderson Cancer Center), Hicks and Wigler demonstrated that gene copy number data on a small number of single cells sampled from different locations in several breast tumors accurately reflected the irregular genomes of the corresponding primary cancers, replete with chromosomal breakpoints and gene copy number variations.

Many of the breast cancer samples scrutinized by the team consisted of several distinct subpopulations of genetically aberrant cells. The team can ferret out and individually characterize each subpopulation. In 2011 they used single-cell sequencing to show that many breast tumors evolve "clonally," in a few punctuated, staccato-like bursts — as opposed to very gradually, bit by irregular genomic bit, as some have supposed. [see illustrations, above]

A clone is a group of genetically identical cells that share a common ancestor. From a single clonal population of aberrant cells, cancers are revealed in single-cell sequencing to advance — and thus enhance their chances of survival — by capitalizing on the process of mutation, which is always occurring, but at a quickened pace in cancer as tumor cells seek new resources to support their continued growth and expansion.

Cells that manage to mutate so as to circumvent threats to their survival — the body's immune cells or poisonous anticancer drugs — gain a survival advantage. Such cells can form the basis of a newly resistant clonal subpopulation within the tumor and seed continued growth.

By exposing clonal subpopulations and inferring their mutational history, Wigler and colleagues have devised a new way to gauge prognosis, while laying bare the specific genetic abnormalities that drive the cancer forward. This can inform treatment decisions and the search for new treatment targets.

Mike Wigler is enthusiastic about what single-cell analysis will be able to do, but he is also emphatic about what it cannot be expected to do. "These methods are, as I said, transformative. But not because they provide answers; rather because they provide a tool to answer questions that couldn't get asked before."

**Peter Tarr**