Sequencing cells one at a time offers singular insight into cancer

COLD SPRING HARBOR, NEW YORK — At last count, the international research community had sequenced close to 4,000 individual cancer samples, with plans in the works to study at least 21,000 more tumor genomes by the end of 2014. These genomic data sets, which span some 50 different cancer types covering nearly every organ in the body, have opened the door to a number of new targeted treatment options by identifying major genetic changes that distinguish cancer cells from normal cells. To date, however, almost all cancer genomes have come from bulk tumor samples containing many different cells, and this cellular lumping can mask some of the fine-grained complexity of the disease that underpins much of drug resistance and susceptibility.

"The best way to begin studying populations of cancer cells is to look at what happens in single cells," says Timour Baslan, a geneticist at the Cold Spring Harbor Laboratory (CSHL) in Long Island, New York, who published a protocol last month for how to analyze copy number variation of single cancer cells (*Nat. Protoc.* **7**, 1024–1041, 2012).

To enable cancer genomics at the individual tumor cell level, in December 2010 Life Technologies, based in Carlsbad, California, announced a $1 million prize to the first team that could sequence the entire genome and RNA content of a single cancer cell. And now, many researchers are getting close to that goal.

At the CSHL Meeting on The Biology of Genomes here last month, Nicholas Navin, a geneticist at the MD Anderson Cancer Center in Houston, presented new data on the genetics of breast cancer at single-cell resolution. Previously, Navin had looked at copy number variants in 100 individual cells in each of two human breast cancer cases and showed that one such tumor contained three distinct subpopulations of cells, whereas the other cancer was made up of a genetically identical pool of cells (*Nature* **472**, 90–94, 2011). Now, Navin has honed his technology to make it sensitive enough to detect single nucleotide differences, and he has found in a set of four cells from a single invasive breast cancer tumor that the individual cells often contain dozens of rare mutations that typically go undetected by bulk tumor-sequencing methods.

"It's my hope that these new tools can be used to improve diagnosis and treatment," Navin says.

"They could become very powerful at detecting remaining cancer cells that can linger in the body for months or years," as well as guide the appropriate course of adjuvant or chemotherapy, he notes.

Of a single mind

Meanwhile, Baslan and others at CSHL scanned for copy number variation in100 cells each from 12 breast cancer tumors both before and after chemotherapy. Reporting at the meeting on the first three such tumors, the researchers found that some people's cancers had gained genetic material after the treatment, whereas other tumors showed signs of chromosomal rearrangements. Although such analyses are possible with bulk sequencing, "this type of evaluation has been traditionally overlooked in cancer studies," says Baslan.

Cancer drug's survivin suppression called into question

There are no prizes for guessing the cellular function of the protein survivin. It was named for its ability to promote cell survival in cancer by blocking programmed cell death—and it's a protein that has become steadily more familiar in the cancer field in recent years. Since its discovery in 1997, survivin has emerged as one the most commonly found proteins in tumor cells. Take into account its rarity in healthy tissue, and you can understand why several companies have invested heavily in putative antisurvivin agents, with a handful already in clinical testing. The lead compound—YM155 from Japan's Astellas Pharma—has already completed phase 2 trials for various forms of cancers. However, new data are emerging from preclinical tests to suggest that the drug might not be working exactly as first anticipated.

The first hint of trouble came in April, when researchers from the Biological Testing Branch of the US National Cancer Institute's Developmental Therapeutics Program in Bethesda, Maryland performed a barrage of *in vitro* tests on YM155, known chemically as sepantronium bromide, and on a similar in-house compound called NSC80467. They found that both agents kill cells primarily by inducing DNA damage, not by suppressing survivin directly—a finding that suggests that any effect on survivin is probably a secondary event caused by general deactivation of gene expression (*Cancer Chemother. Pharmacol.* doi:10.1007/s00280-012-1868-0, 2012).

Then, last month, a team from the University of Pittsburgh School of Medicine published the results of a large chemical screen for small molecules that could kill an aggressive form of skin cancer caused by the Merkel cell polyomavirus. Of more than 1,300 compounds tested, including many standard chemotherapeutic drugs, only YM155 was able to destroy Merkel cell carcinomas, yet not for the reasons suspected (*Sci. Transl. Med.* **4**, 133ra56, 2012).

"We anticipated that YM155 would cause apoptosis; instead, we saw it causes a profound inhibition of DNA synthesis," says study author Patrick Moore, director of the Molecular Virology Program at the University of Pittsburgh Cancer Institute. Indeed, notes Bill Chang, a pediatric oncologist at the Oregon Health & Science University in Portland who was not involved in the work, "we still do not quite understand the mechanism of survivin inhibition, and, further, any drug that inhibits expression of survivin may have off-target effects."

Mechanistic melee

The findings contradict Astellas's own preliminary data, which suggested that "YM155 is not a DNA-damaging agent," says Aya Kita, an Astellas scientist who has led the drug's development.

Regardless of the drug's mechanism of action, the company is forging ahead with its clinical development of YM155—currently the only small-molecule drug in human testing that purportedly blocks survivin expression. YM155 has already been tested as a single agent to treat patients with melanoma, lung cancer, prostate cancer or lymphoma. Although the drug seems to be safe and well tolerated, its efficacy to date has been modest at best. In a recent study of 41 people with diffuse large B cell lymphoma, only one person had a complete remission and an additional two participants had a significant response after the therapy (*Cancer* doi:10.1002/cncr.26510, 2011).

Those findings don't worry Kita, who insists that the real test will come when the drug is combined with other therapies to simultaneously inhibit different cell-survival pathways. In cell culture and mouse models, Kita and her colleagues have shown that YM155 in combination with rituximab, a

Baslan and his colleagues started out by studying copy number variation because this method requires less starting material than is needed for full sequencing—and single cells don't have much DNA to begin with. In March, however, scientists from the BGI genomics center in Shenzhen, China reported a way to amplify the DNA three-million-fold, creating enough starting material to decode the entire protein-coding portion of the genome down to the single nucleotide level for individual cancer cells (*Cell* **148**, 873–885, 2012).

Future single-cell sequencing projects should also become easier thanks to the creation last month of a new single-cell genomics center at the Broad Institute in Cambridge, Massachusetts, with equipment and staff support from Fluidigm Corporation, a microfluidics technology company based in South San Francisco. According to Fluidigm's president and chief executive Gajus Worthington, the new center will begin by focusing on cancer stem cells and circulating tumor cells. "We hope that studying these cells will lead to a new type of biopsy that identifies from the start whether a tumor will spread or is drug resistant," he says.

Jeanne Erdmann

---

monoclonal antibody directed against the CD20 protein found on the surface of B cells, results in significant improvements in tumor regression and survival (*Leuk. Res.* **35**, 787–792, 2011). Trials of YM155 in combination with carboplatin plus paclitaxel chemotherapy to treat patients with lung cancer and other solid tumors are ongoing.

Meanwhile, alternative strategies to suppress survivin have also entered early clinical testing, including vaccines to generate an antigen-specific immune response against tumor cells that express survivin and several antisense oligonucleotides. The latter—which include products from Indiana's Eli Lilly, New Jersey's Enzon Pharmaceuticals and Denmark's Santaris Pharma—should in theory be able to silence the *BIRC5* gene, which codes for survivin, with more precision than small molecules such as YM155.

Nonetheless, Anthony Tolcher, director of clinical research at South Texas Accelerated Research Therapeutics in San Antonio, who has led trials involving both YM155 and antisense therapies, expects that delivery challenges associated with getting antisense molecules into the target cancer cells will most likely mean that YM155 or another small-molecule drug yet to enter the clinic will be the first such agent to hit the market. Whether or not that drug hits survivin directly, however, remains to be seen.

David Holmes

A history of drugs on the weight list

Antiobesity medications have a weighty history in the US. Throughout the post-war era, amphetamines were widely touted for their appetite-suppressing effects until their addictive potential prompted the US Food and Drug Administration (FDA) to ban their use as a weight-loss aid in 1979. The mid-1990s then saw the surge in popularity of fen-phen—a combination of the two anorectics, fenfluramine and phentermine—before that diet pill, too, was pulled from the market due to concerns over serious heart valve problems. More recent attempts haven't fared much better: Meridia, Acomplia and Ephedra all had to be yanked from pharmacy shelves owing to spotty safety records.

Will the latest obesity remedies—Lorqess (lorcaserin) and Qnexa (a combination of phentermine and topiramate)—stand the test of time? In 2010, the FDA, worried about dangerous side effects, rejected both agents. But in February, FDA advisors reviewed new data and recommended granting market approval to Qnexa, a drug from Mountain View, California–based Vivus that combines an appetite suppressant and an antiepileptic, as an obesity treatment. And last month, the same committee ruled in favor of licensing Lorqess, a serotonin receptor agonist from San Diego–based Arena Pharmaceuticals.

If the FDA follows its panel's advice—decisions are expected before mid-July—these drugs would be the first new prescription antiobesity pills to reach the US market in 13 years. Here we give the skinny on the recent checkered past of weight-loss drugs.

Elie Dolgin

Nov 1997

Abbott's Meridia (sibutramine), a neurotransmitter reuptake inhibitor, wins FDA approval.

Meridia was withdrawn from the US market in October 2010 after clinical trials revealed an increased risk of heart attack and stroke.

Apr 1999

Roche's Xenical (orlistat), which reduces intestinal fat absorption by inhibiting pancreatic lipase, receives FDA approval. It remains the only prescription antiobesity medication approved for long-term use.

Apr 2004

The FDA bans the sale of ephedra-containing dietary supplements, citing heart-rate and blood-pressure abnormalities, leading to strokes and sudden deaths.

Jun 2006

The European Medicines Agency approves Sanofi's cannabinoid receptor blocker Acomplia (rimonabant), a decision the agency reversed in 2009 owing to psychiatric risks. The FDA never approved Acomplia.

Feb 2007

The FDA approves GlaxoSmithKline's Alli, an over-the-counter formulation of orlistat.

Oct 2010

The FDA rejects both Lorqess and Qnexa. Less than two years later, advisory panels recommend in favor of approving both medicines, arguing that the benefits outweigh the risks.

Feb 2011

Despite an earlier advisory panel's recommendation in favor of approval, the FDA rejects Orexigen Therapeutics's Contrave, a combination of the antidepressant bupropion and the opioid receptor blocker naltrexone, citing concerns about possible cardiovascular risks.

Feb 2012

The FDA's Endocrinologic and Metabolic Drugs Advisory-Committee (EMDAC) votes 20–2 in favor of approving Qnexa.

May 2012

The EMDAC backs Lorqess in an 18-4 vote.