Fred Hutch Study Finds Genetic Driver of Drug Resistance in Small Cell Lung Cancer

Researchers use CRISPR-Cas9 gene-editing tool on tumor cells grown in mice to better model how tumors that initially respond to chemotherapy become resistant.

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Small cell lung cancer, or SCLC, is aggressive, lethal and particularly cruel because chemotherapy initially works so well.

But within just a few months, SCLC becomes resistant to drugs and dashes hopes.

“What is seen in the clinic is often quite remarkable responses to chemotherapy initially, but these are just transient responses and tumors come back,” said David MacPherson, PhD, a Fred Hutch Cancer Center scientist who specializes in small cell lung cancer. “The vast majority of patients — despite having striking initial responses — do extremely poorly.”

The five-year survival rate for SCLC is less than 6%.

MacPherson’s lab in the Human Biology Division investigates what causes this rapid switch from hope to despair in SCLC, which accounts for about 15% of lung cancers.

A study from the lab published recently in the journal Science Advances describes a new way to screen for the genes that matter in chemotherapy resistance by using human tumor cells implanted in mice.

The study identifies a gene that is well understood in other cancer types — but poorly characterized in SCLC — that drives drug resistance when it gets turned off, which could lead to new therapies and improved odds for SCLC patients.

PDX mice aren’t from Portland

Researchers often manipulate genes in cancer cells grown in laboratories under controlled conditions to understand what role they play in disease, but those cell lines only partly mimic how

cancer behaves in a living organism.

SCLC cell lines grown on plastic and exposed to drugs in experiments don't reliably re-enact the rapid switch to chemotherapy resistance observed in patients, which limits their value in understanding the genetic mechanisms driving that lethal transition.

However, mice implanted with human tumor cells that have not been grown on plastic — patient derived xenograft (PDX) models of the disease — show the same response to chemotherapy as the patients from whom the samples were collected.

MacPherson, who holds the Vinh Bui and Tram Le Endowed Chair for Lung Cancer, uses SCLC cells floating in patients' blood to set up PDX mouse models for experiments that provide a more authentic environment than a lab dish.

For example, MacPherson's lab has previously shown that when they altered (scientists say "perturbed") the MCYN gene, causing it to produce more of its product than usual in PDX models of SCLC, they observed the switch to chemotherapy resistance.

"What we wanted to do with this study is move from single-gene perturbations to querying hundreds or thousands of genetic perturbations to identify which ones of those are important," MacPherson said.

One way to perturb genes is to knock them out of commission and see what happens in their absence.

Making a screen with guides and scissors

The team used a molecular tool called CRISPR-Cas9 to knock out genes researchers believe could play a role in making tumors resistant to chemotherapy.

This versatile, Nobel Prize-winning tool has two parts: an enzyme called Cas9 that snips DNA at precise locations and CRISPR guide molecules that deliver the Cas9 snippers to the gene that researchers want to knock out.

When the cell repairs the break, it's usually not good enough to restore the gene's function, which knocks it out.

The CRISPR guides can be combined in a library of gene knockouts that can be applied to a population of cells in a single experiment.

MacPhersons' team built a library of CRISPR guides for 400 genes that might play a role in making tumors resistant to chemotherapy.

"Our goal was to identify genes that were important for this switch from small cell lung cancer being exquisitely chemo-sensitive to rapidly becoming chemo-resistant," MacPherson said.

They converted the library into viral particles capable of infecting cells so that each cell receives just one CRISPR guide directing Cas9 where to snip, which knocks out one — and only one — gene per cell.

By dripping the viral knockout brew on tumor cells, they could perform experiments to see which cells live and which cells die under drug treatment conditions, screening out irrelevant genes to identify the genes that matter most for survival.

Modeling knockouts in mice

CRISPR screens often are applied to tumor cells grown outside of their native environments in plastic dishes.

But that's the approach that hasn't worked so well for SCLC because the cells don't mimic how tumors behave in living organisms when they receive chemotherapy.

So instead, MacPherson's team tested their library of knockouts on tumor tissue growing in PDX mice.

First, they removed tumor cells from the mice and exposed them to their viral library of CRISPR guides.

They reserved some of the transformed cells to represent conditions at the beginning of the experiment. Then they re-implanted the rest of the transformed cells (about a million cells per mouse).

Once the mice developed tumors, they were exposed to one of two conditions.

Some mice received chemotherapy and others received only saline. The mice that received chemotherapy showed a similar response as the human patients: tumors shrank, but then they came back.

The team removed tumors from both groups and analyzed the tissue with genomic sequencing to see which of the knocked-out genes mattered in the tumors that came back.

Their screen turned up some of the usual suspects in SCLC, but it also spotlighted a new driver of chemotherapy resistance: a gene called KEAP1.

When they knocked out KEAP1, tumor cells that had been vulnerable to chemotherapy became resistant.

Clinical relevance

Cells turn off KEAP1 when they respond to stress caused by substances called oxidants that snatch

electrons from other molecules and damage cells. Turning off KEAP1 activates other molecules in an antioxidant signaling pathway that repairs the damage.

It's an essential cellular process for survival, but if that pathway stays activated too long, it can fuel tumor growth.

When KEAP1 popped up in the screen, it got the researchers' attention because KEAP1 dysregulation is also known to confer chemotherapy resistance in the most common type of lung cancer, though its role in SCLC has not been well characterized.

Tumors linked to KEAP1 mutations in other cancers also are known to depend on a process that breaks down an amino acid called glutamine to produce energy supporting rapid growth and proliferation.

Co-first author, Lauren Brumage, PhD, performed an experiment to see if that dependence on glutamine metabolism also exists in SCLC tumors when KEAP1 is knocked out.

Her experiment confirmed that loss of KEAP1 creates the same vulnerability and potential for a drug that would inhibit something the tumor needs to grow.

"This mutation on the one hand causes this dramatic chemoresistance, but it also allows for a therapeutic vulnerability that could be exploited in the clinic, potentially," MacPherson said.

Brumage, then a student in the Molecular & Cellular Biology Graduate Program jointly offered by the University of Washington and Fred Hutch, did much of her doctoral work in the MacPherson lab. Co-first author, Scott Best, also is a student in the same program.

Brumage earned her PhD last year and is now a scientist working for the Seattle-based biotech company Omeros, which develops small-molecule and antibody therapeutics.

The MacPherson team received further confirmation that they were on to something clinically relevant when they collaborated with Barzin Nabet, PhD, a scientist with the South San Francisco-based biotech company Genentech.

He analyzed data from a Phase III clinical drug trial for patients with extensive stage SCLC and found that 6% of the patients had mutations to KEAP1 in their tumors, and the tumors in other patients found different ways to activate the same program caused by KEAP1 mutation. Activation of this program correlated with worse responses to treatment and worse overall survival.

"It speaks to the real-world relevance of what we're doing in these mouse models," Brumage said. "I was just glad to see that there is actual clinical significance to this because that's really what got me into science and research in the first place."

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