Researchers discover molecular fail-safe that keeps bladder tissues from turning cancerous

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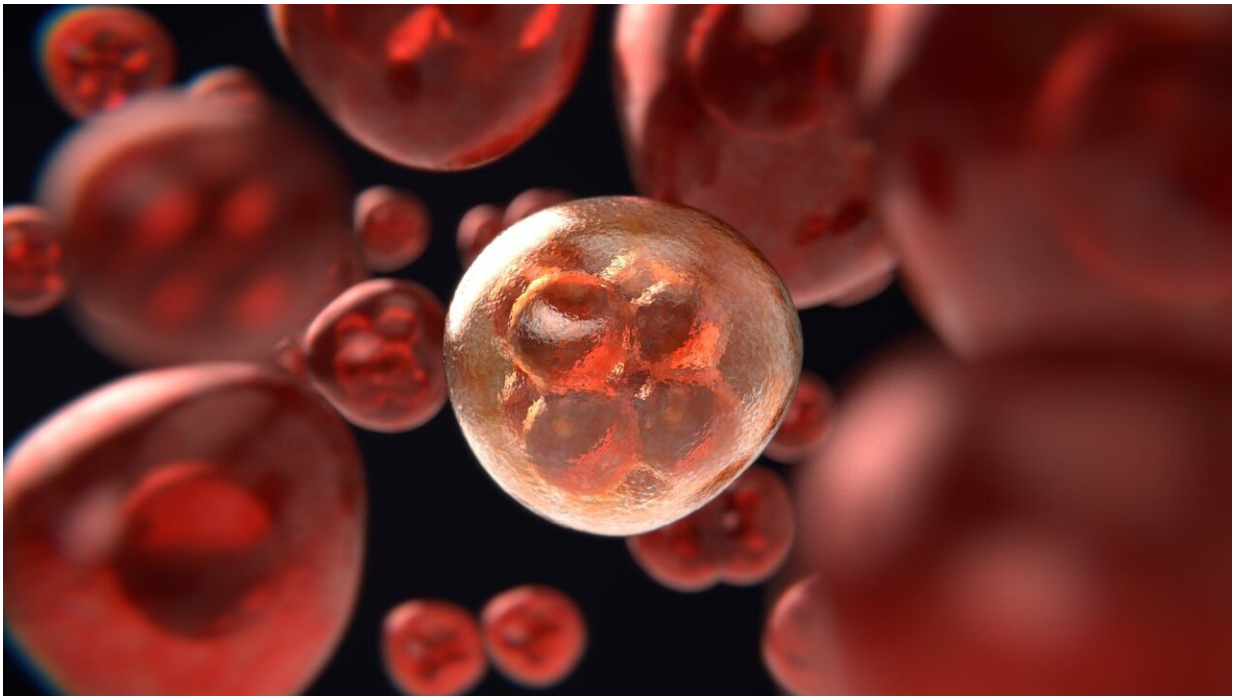

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Over the decades, scientists have revealed many strategies our cells use to keep normal cells from becoming cancerous. Now, Fred Hutchinson Cancer Center scientists have discovered a completely new—and counterintuitive—emergency brake that bladder cells use to stave off tumors even when cancer-promoting genes are turned on.

In a study published April 20 in *Cancer Cell*, the team also manipulated the brake to halt the growth of human bladder tumors in mice, suggesting that the newly discovered molecular fail-safe could be a target for future bladder cancer therapies.

"We think what we have defined is a new tumor-suppressive mechanism," said Andrew Hsieh, MD, the Fred Hutch physician-scientist who studies bladder and prostate cancers and treats patients with these diseases. "We've also showed that in preclinical models of bladder cancer we can reinstall this emergency brake."

Sujata Jana, Ph.D., a postdoctoral fellow in Hsieh's lab, found that mutation of a key tumor-suppressing gene, called ARID1A, in bladder cells ramps up a suite of cancer-causing genes. At the same time, the mutated cells ramp down a molecule needed to turn these genes' messenger RNAs into the cancer-causing proteins that enable unrestrained growth and survival.

Jana and Hsieh dubbed the newly discovered emergency brake "transcriptional-translational conflict" for the opposing forces at work: the increased transcription of genes that could drive cancer, countered by the decreased translation of their proteins. Tumors missing ARID1A have overcome this conflict, but the scientists showed that genetic or pharmacological strategies that reinstate it can dramatically slow tumor growth.

Not all bladder tumors have lost ARID1A activity, but the findings suggest that tumors with ARID1A mutations may have a unique—and potentially targetable—sensitivity to reductions in protein synthesis.

"And if this finding holds true in other cancers, it is going to help us understand better how to target the translation apparatus," Hsieh said. "That will be important as more inhibitors of translation arise, and our

genetic understanding of cancers becomes very, very solid."

Better treatment options needed for advanced bladder cancer

Scientists estimate that about 82,300 people in the United States will be diagnosed with bladder cancer in 2023. About three times as many men as women get the disease, and most people are diagnosed after age 55.

Patients with late-stage disease have fewer effective treatment options and there is less known about their tumors. With Fred Hutch and University of Washington colleagues, Hsieh created the Fred Hutch/UW bladder cancer rapid autopsy program to address this disparity. Generous patients who participate in the program donate metastatic bladder tumor tissue to help researchers better understand—and target—the deadly disease.

Revealing the hidden meaning behind an unexpected result

When Hsieh joined Fred Hutch in 2014, our understanding of how bladder cancers grow was even more limited, he said. He decided to start a new line of research to help expand scientists' understanding of the disease. Surveying mutations that may contribute to bladder cancer, Hsieh noticed that ARID1A is one of the most frequently mutated genes, suggesting that it usually works to suppress tumor formation in some way. In healthy cells, ARID1A helps regulate whether genes are turned on and off by changing DNA packaging and its molecular modifications.

Jana took up the challenge of figuring out how ARID1A suppresses bladder tumors and why mutations that block its activity help promote tumors. But when she deleted ARID1A in the cells lining the bladder,

mice did not develop tumors even after 400 days—even though they also ramped up a suite of pro-growth genes.

The findings began making sense when Jana considered another unexpected finding in bladder cells missing ARID1A: RNA translation (or protein synthesis) went down in these cells, not up.

"Tumor suppressors have long been considered negative regulators of translation," Hsieh said. Cancer cells need a lot of new proteins to grow, survive and create more cancer cells. "The idea was that you need more translation for cancer to occur, so it was thought that the tumor suppressor state was one of decreased translation, and that's how the field looked at it for many years."

The unexpected results weren't negative findings, Jana said, they were clues: "It felt like we were communicating with the cells in a language we didn't know. When we got a curveball, we tried to figure out, 'What does it mean?'"

She and Hsieh realized that to decipher the new cellular language, they needed to set aside their preconceived notions about how tumor suppressors work. They realized that the pro-cancer genes turned on in cells lacking ARID1A were like a pile of dry tinder waiting for a match. As long as the cells kept the match—RNA translation—out of reach, no tumors could ignite. Jana and Hsieh had discovered a secondary safety hatch that is only triggered when the main safety valve—ARID1A—fails.

Using a combination of pharmacological approaches and genetically modified mice, Jana was able to outline the sophisticated molecular interplay that link loss of ARID1A to reduced protein synthesis. Loss of ARID1A ultimately slows the speed at which protein-making machines move along RNA transcripts and reduces output of new proteins.

"That was remarkable," Hsieh said. "Transcriptional-translational conflict links chromatin remodeling with the speed of translation across coding sequences."

Overcoming hurdle clears the way for tumors

Jana used various methods to confirm that translational-transcriptional conflict suppresses tumor formation. Notably, she found that a bladder cancer-promoting compound in cigarette smoke, called BBN (for N-butyl-N-(4-hydroxybutyl)nitrosamine), boosts the levels of the protein machine component blocked by ARID1A loss, and an uptick in protein production.

This suggested that BBN could help cells get over the hurdle posed by transcriptional-translational conflict. They were right: dosing mice with BBN prior to deleting ARID1A balanced translation and unleashed the pro-cancer potential lurking in the genes turned on without ARID1A. Tumors grew and progressed more quickly in BBN-treated mice lacking ARID1A than in mice that retained the gene.

Jana saw that bladder tumors from people also appeared to have overcome the conflict. Tumors with low levels of ARID1A were more likely to have evidence of higher protein production—suggesting that like the BBN-caused tumors in the mice, these tumors had evolved strategies to overcome transcriptional-translational conflict. And ARID1A mutations in bladder tumors don't show up alone; they're usually accompanied by changes in genes that regulate RNA translation.

"Because cancer is so smart, it's devised ways around this transcriptional-translational conflict," Hsieh said. "It's figured out, 'Okay, if I just boost up translation first, then this whole problem is gone.'"

A new therapeutic vulnerability

But what if scientists could make this "conflict" a problem again for bladder tumors? Hsieh and Jana saw an opportunity to reinstall the emergency brake.

Jana used a compound called homoharringtonine, or HHT, which blocks RNA translation. A clinical version of HHT, called omacetaxine mepesuccinate, has already been approved by the Federal Drug Administration to treat chronic myeloid leukemia. Jana used tissue from BBN-induced tumors to create 3D dish-based tumor models called organoids. HHT slowed the growth of organoids missing ARID1A at concentrations that didn't stop organoids with normal ARID1A from growing. She saw similar results in bladder cancer cells taken from human tumors.

Jana then turned to patient-derived xenografts, or PDXs, models of bladder cancer. HHT decreased tumor growth by 59% in PDX model with low levels of ARID1A and by 36% in the model with moderate levels of ARID1A, but did not affect tumor growth in the PDX line with high levels of the protein.

Next steps: More biology, clinical application

"This work shows that there is a dynamic interplay between transcription and translation that's actually functional," Hsieh said. "And this is the first, we think, of many examples that will be discovered."

ARID1A appears to act as a tumor suppressor elsewhere, but it's not yet known whether tissues like the ovary and liver also rely on transcriptional-translational conflict as an emergency brake, he said. Hsieh's team is developing technologies to delve even deeper into the

phenomenon and the potential importance of translation speed in cancer.

"The paper really expands our understanding of basic cancer biology, but also it provides a potential therapeutic window—or a deeper understanding—of how we could use this genomic alteration [in ARID1A] as a foothold for treating patients with advanced disease," Hsieh said.

More information: Andrew C. Hsieh & collegaues, Transcriptional-translational conflict is a barrier to cellular transformation and cancer progression, *Cancer Cell* (2023). DOI: 10.1016/j.ccell.2023.03.021. www.cell.com/cancer-cell/fullt … 1535-6108(23)00094-6

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