RNA stability may play a role in prostate cancer

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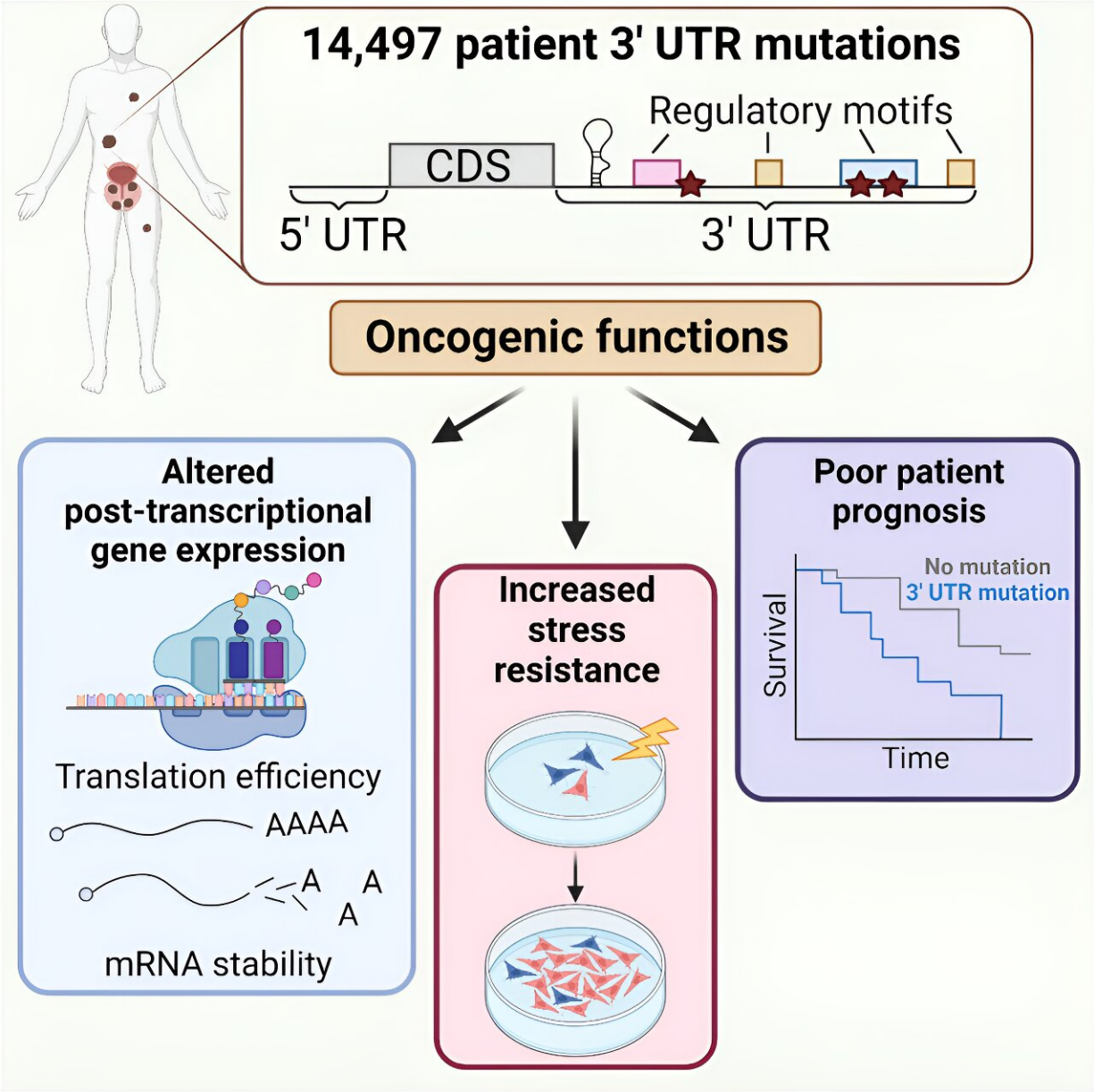

Graphical abstract. Credit: *Cell Reports* (2023). DOI: 10.1016/j.celrep.2023.112840

Mutations in a genetic region that regulates RNA stability could influence prostate cancer outcomes and drug resistance, according to new work from scientists at Fred Hutchinson Cancer Center published in *Cell Reports*.

"The basic takeaway is that the lifecycle of an RNA [molecule] is really important for prostate cancer—and it's functionally associated with patient outcomes," said Fred Hutch prostate cancer expert Andrew Hsieh, MD, the study's senior author.

The team developed innovative new techniques to test whether cancer-associated mutations in a little-studied and still mysterious region of RNA could affect protein production and cellular function.

Samantha Schuster, Ph.D., while a graduate student in Hsieh's lab, developed two complementary methods that allowed her to assess the significance of thousands of cancer-associated mutations that have been found lurking in DNA of advanced prostate tumors. Her work suggests that mutations in this key genetic region can affect RNA in ways that could help cancer cells grow and resist drug treatment.

As scientists strive to add to the arsenal of precision oncology strategies and tailored cancer treatments, Schuster and Hsieh hope the study will encourage these researchers to think more expansively about which mutations may contribute to cancer, and where to hunt for future therapeutic targets.

"What our findings say is, this area is important—look here," Schuster

said.

RNA: an essential link between gene and protein

COVID-19 RNA-based vaccines first introduced messenger RNA, the intermediate molecules that make it possible for our cells to turn information contained within DNA into proteins, to a global audience.

Proteins run our cells' biological processes, and the instructions for constructing them are embedded in our DNA. To build proteins, our cells copy, or transcribe, this information into many molecules of messenger or mRNA, which carry it to protein-building molecular machines that translate it into strings of amino acids.

A lot of factors regulate how many proteins get produced after a gene is turned on.

"An important thing to understand is that each of those steps is not a linear pathway," Schuster said. "There are so many weird other regulatory steps that can exist. … And any of those can mess up proteins, which can lead to cancer."

How many mRNAs are made, how long they last, and how many proteins are produced off each copy—all play a role, and lot of this is regulated by the mRNAs themselves.

Schuster focuses on a section of RNA that doesn't encode protein information, but instead helps regulate protein synthesis and how long an RNA sticks around before it gets broken down. It's one of the segments known as an untranslated region, or UTR, at each end of an mRNA.

At one end is the 5' (or 5-prime) UTR, and at the other is the 3'UTR. (RNA is made up of ribonucleic acid bases, which are essentially rings

of five carbon atoms. The carbon atoms are labeled from 1 to 5 and bases link together by using carbons 5 and 3 to a shared phosphate group. At one end of the RNA strand the carbon 5 is unattached, and at the other end, it's carbon 3 that's free.)

UTRs can also be mutated, which could alter protein levels even if the proteins produced are normal. Excess growth is a hallmark of a cancer. Too much of a growth-enhancing protein, or too little of a growth-restraining protein, could make cells become or stay cancerous.

Hsieh's team has already showed that prostate cancer-associated mutations in the 5'UTR likely play an important role in prostate cancer. That left a question mark hanging over mRNA's other UTR.

"A lot of attention has been paid to a few individual mutations in the 3'UTR, but not much attention has been paid to the 3'UTR itself. It's a blind spot," Schuster said.

It's also a technical headache. Like DNA, RNA is made up nucleotides (also known as bases). Both UTRs can vary in their number of bases, but the 5'UTR tops out at about 1600. The 3'UTR can be three times as long—too long for even our most advanced sequencing technologies to complete in a single step.

To study the effects of thousands of prostate cancer-associated 3'UTR mutations, Schuster needed to develop techniques that tackled long 3'UTRs in a multiplexed fashion. She carefully adapted what's known as a massively parallel reporter assay, or MPRA, to overcome the challenge of long 3'UTRs and examine their two main roles. One MPRA allows her to examine how mutations affect translation efficiency (the rate at which proteins are produced from an mRNA), while the second MPRA technique assesses RNA degradation and stability.

"Her goal was to get to, "What is the action of these [mutations] on the cell?" And that has not been done," Hsieh said. "It's a testament to her scientific grit."

Cancer-associated 3'UTR mutations may promote growth, drug resistance

Schuster applied her methods to more than 14,000 3'UTR mutations found in 185 advanced prostate tumors that have spread and developed resistance to androgen-blocking treatments, also known as metastatic castration-resistant prostate cancer. She examined mutations present in tumors but not in matched samples of healthy tissue.

The samples came from 79 patients who had donated tissue to the University of Washington Tissue Acquisition Necropsy program; 101 patients who had donated to the Stand Up to Cancer West Coast Dream Team project; and five patient-derived xenograft models (in which tumor tissue taken from patients is grown in mice).

When she applied her MPRA approach to the 14,000 3'UTR mutations, Schuster saw that many altered translation efficiency. Functional mutations in pro-cancer genes typically enhanced protein production—up to 16-fold. Those in anti-cancer genes usually reduced translation efficiency, even as much as three-fold, which would lead to lower levels of a potentially cancer-inhibiting protein.

When she assessed RNA stability, Schuster found that 150 patient-based 3'UTR mutations, many in genes known to promote cancer, changed RNA stability. Most occurred in genes that may help prostate cancer shift to a neuroendocrine type, which can arise as tumors evolve to escape hormone-blocking therapies.

Schuster used CRISPR to introduce two of the 3'UTR mutations that had the biggest effects on protein production into DNA to test whether they also affect cellular function. At least one, in a gene for the protein ZWILCH, which plays an important role in cell division, may be able to help tumor cells overcome cisplatin exposure. Cisplatin is a chemotherapy that damages DNA and blocks cell division right at the point where ZWILCH is most important.

"This is the first time ever that anyone has shown that a patient-based mutation in the 3'UTR may enable drug resistance," Hsieh said.

The other mutation Schuster tested helped cells grow despite stressful, low-nutrient conditions, similar to those often found within tumors.

When prostate cancer patients had these mutations in their tumors, the tumors became resistant to hormone-blocking treatments more quickly, spread through the body more quickly, and had shorter survival times.

Next: digging deeper into RNA biology, cancer

In addition to demonstrating that 3'UTR mutations could have potentially cancer-promoting effects on genes, Schuster's findings suggest that mutations in 3'UTRs could teach researchers more about how the RNA sequence and shape influence how it works and is regulated. More than 75% of 3'UTR mutations that affected translational efficiency changed sequences other molecules use to interact with the RNA.

Importantly, many of the mutations don't just affect RNA sequence—they affect its shape, said Schuster. RNA is a single strand of bases that can fold back on itself and form complex and dynamic shapes, which influence how it interacts with RNA-regulating proteins and the protein-synthesizing apparatus.

"These areas are basically molecular handles for proteins or other RNA. And it's those interactions that allow RNAs to persist or be degraded," Hsieh said. "And by studying how cancer-associated mutations affect that, we can get a better sense of what's important."

He and his team are very interested in understanding how interactions between proteins and RNA work, and how to disrupt them—a step toward developing treatments that may be able to target them.

Hsieh also hopes to better understand how RNA interacts with RNA, as the 3' and 5'UTRs also interact with each other in potentially important ways. And while messenger RNAs might be the best-known, there are many other types of RNAs in cells, and some bind to and regulate 3'UTRs.

Another future step will be to look at the genetics of 3'UTRs in individual cells, Hsieh said. He suspects that mutations in 3'UTRs may help individual cancer cells succeed in the shifting environment of a tumor, initiating new avenues of evolution that can induce drug resistance or help them grow in less-than-optimal conditions. They're an important part of the mutational landscape of prostate cancers—and perhaps other types of tumors as well.

"The landscape is not a static thing. The functional effects [of mutations] create new contours. It's the functionality of the landscape that creates the tumor ecosystem," Hsieh said. "This is the first paper to look at that whole spectrum within prostate cancer. And because of that, I think we have a good look at how these mutations actually function."

More information: Samantha L. Schuster et al, Multi-level functional genomics reveals molecular and cellular oncogenicity of patient-based 3′ untranslated region mutations, *Cell Reports* (2023). DOI: 10.1016/j.celrep.2023.112840

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