

New Methods Reveal Cancer Mechanism in Ancient Genes

Fred Hutch researchers discover that overproduction of DNA packaging material predicts aggressive brain and breast tumors, which could lead to cheaper diagnostic tests and new drug therapies.

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Researchers at Fred Hutch Cancer Center have discovered an overlooked mechanism driving aggressive breast and brain tumors involving genes so ancient — more than 2 billion years old — that they fly under the radar of standard genetic sequencing methods.

“Identifying this mechanism suggests it could be a new test to diagnose cancers and possibly treat them,” said Fred Hutch molecular biologist [Steven Henikoff, PhD](#), co-first author of a study recently [published in the journal Science](#).

The study focuses on 64 ancestral genes needed to make histones, which are the molecular packaging material that helps squish some six feet of DNA strands into a single cell’s nucleus.

His team discovered that the overproduction of histones — measured by the increased presence of an enzyme that kick-starts the process — predicted the aggressiveness and likelihood of recurrence after surgery in preserved brain and breast tumor samples from patients whose clinical histories are documented.

The discovery reveals a new biomarker to aid in the early detection of disease and a potential target for more precisely tailored therapies — made possible by a collaboration between labs in different research divisions that defines the scientific culture Fred Hutch has nurtured for half a century.

Making the most from patient tumor samples

The histone study began with a phone call between Henikoff in the [Basic Sciences Division](#) and co-author [Eric Holland, MD, PhD](#), a Fred Hutch brain cancer researcher who heads the [Human Biology Division](#) and holds the Endowed Chair in Cancer Biology.

Part of Fred Hutch’s commitment to building preeminence in precision oncology — diagnosis and

treatment tailored to a patient's individual biology — involves research that requires extracting molecular information from preserved patient tumor samples.

Cross-referencing that molecular information with patient medical records enables researchers to match genetic profiles to clinical outcomes and classify tumors based on their biology, which is more accurate than simply lumping them together based on how they look under a microscope.

Though all human cells share the same DNA, each kind of cell — such as brain, skin and kidney — requires different genes to be turned on or expressed at different times depending on that cell's function. Many things can go wrong in that process that may turn cells cancerous.

Gene expression begins in the cell's nucleus with transcription, which makes RNA copies of genetic sequences from DNA, which serve as templates. Those copies are delivered in the form of RNA molecules to factories in the cell that make proteins, the cell's molecular workers. The factory reads the RNA template, translating the genetic sequences into the amino acid sequences needed to make each protein.

Holland explains it like this: Imagine that a cell's nucleus is like the Library of Congress.

The cell's DNA, packaged into chromosomes inside the nucleus, contains all the library's books accumulated over the cell's long evolution on Earth. Those books can never be checked out.

But they can be copied, and those copies can be carried out of the library by RNA molecules.

A standard method of mining samples for molecular information is called RNA sequencing, which tells you what books in the library are getting copied a lot based on how many copies are in circulation outside the library.

From that information, you can figure out which genes got copied inside the nucleus and how often, which reveals patterns of gene expression in healthy cells that may change in cancerous ones.

RNA sequencing works best on fresh-frozen cells, but that's not usually how samples from surgery or biopsies are prepared.

For more than a century, the preferred method for long-term preservation of samples involves fixing fresh tissue in an embalming fluid called formalin and embedding the tissue in paraffin wax. That process creates formalin-fixed paraffin-embedded (FFPE) samples, the most common kind available for research.

Like most hospitals and research institutions, Fred Hutch has plenty of these samples spanning decades, but long-term exposure to the formalin damages the genetic material, making those samples practically unreadable with standard RNA sequencing.

When Henikoff learned that Holland wanted to find ways to mine more molecular information from

their samples, he saw an opportunity for the two labs to collaborate.

“I called him up and asked if he was just going to use RNA sequencing, because we have something that might work pretty well,” Henikoff said.

He remembered Holland joking that he had so many paraffin blocks around the lab he used them as doorstops.

“I decided he’d be fun to work with,” Henikoff said.

Henikoff had developed a faster, cheaper alternative to RNA sequencing that he had tweaked to get molecular information out of paraffin-embedded samples.

Henikoff’s sequencing method is called Cleavage Under Targeted Accessible Chromatin, or CUTAC.

It’s different than standard RNA sequencing because it reveals what is going on at the beginning of the transcription process when DNA is getting copied inside the nucleus rather than at the end when RNA copies already are in circulation.

CUTAC identifies which books are getting copied as it’s happening.

It does this by tracking the activity of an enzyme called RNA polymerase II as it moves across DNA, pausing at various places where it accumulates and kick-starts the copying process, including stretches concerning gene regulation that don’t get picked up by RNA sequencing.

Using CUTAC, the Henikoff and Holland labs were able to distinguish different differences in gene regulation between tumor and normal tissues in mouse brain and liver samples.

“This is a very inexpensive way to get a lot of information out of paraffin sections, and [Steve’s lab](#) developed it,” Holland said.

They [published the results in Nature Communications](#) in 2023.

The next step was to apply CUTAC to multiple cancer types, which led to the current study.

Finding an ancient mechanism

Henikoff and his team used the modified CUTAC method to better understand a common phenomenon in cancer called hypertranscription, which predicts a poor prognosis.

It’s characterized by an overall increase in RNA polymerase II, which turns on thousands of genes, keeping the library’s copy machines in the nucleus working overtime.

But hypertranscription expresses so many genes at the same time that it’s difficult to isolate the mechanism that drives aggressive tumors.

Henikoff figured the most relevant genes to study would be the ones that limit how fast cells can double.

He zeroed in on a subset of just 64 ancestral genes needed to make histones, which are the molecular packaging material that helps squish DNA inside the cell's nucleus.

DNA strands wrap around histones, which are clustered into eight-histone balls that are threaded like beads on a string into fibers that are further intertwined to form chromosomes.

Cells don't store extra histones like cardboard boxes in the garage.

On moving day when cells replicate their DNA and divide, histones must be rapidly produced just in time for the cell to copy its chromosomes and make sure each new cell gets a complete and identical set.

When that happens, there's such an abundance of RNA polymerase II lingering over histone genes in mice and fruit flies during cell replication that levels of the enzyme drop by as much as 40 percent elsewhere.

But the potential role of histones in fueling cancer growth has been overlooked in the field because they fly under the radar of standard RNA sequencing.

When most genes are copied, the RNA molecules carrying the copies to the protein factories acquire a stabilizing modification that gives them a distinct chemical signature, making them detectable by RNA sequencing.

But histone genes are so old they predate the rise of eukaryotic life (organisms with cells that contain a nucleus, which emerged between [1.8 and 2.7 billion years ago](#)).

Their RNA copies use more ancient chemistry to stabilize, and because they lack that common signature, they're like a plane that's invisible to the control tower because it's flying without a transponder.

Henikoff's CUTAC method solves that problem because it tracks which DNA is getting copied at the beginning of the transcription process inside the nucleus instead of the resulting RNA copies at the end of the process.

They hypothesized that the single functional role of hypertranscription in cancer is to crank out enough histones to keep pace with the DNA packaging requirements for tumor cells to proliferate faster than normal.

They tested their hypothesis on a set of 36 human meningioma brain tumor samples from Holland's lab, which are cross-referenced with patients' medical histories.

That enabled them to link tumor biology with patient outcomes using paraffin-embedded samples

already collected and stored.

They discovered that overproduction of histones alone predicted the aggressiveness and recurrence of meningioma tumors. Overproduction of histones also predicted aggressiveness of invasive breast cancer based on an analysis of 13 paraffin-embedded samples.

One of the study's co-first authors, [Ye Zheng, PhD](#), then a postdoctoral researcher in Henikoff's lab, discovered a correlation between elevated histone levels and changes to chromosomes that drive many, but not all cancers.

When cells divide, each of the 46 chromosomes makes a copy of itself, and the copies are hinged by a structure called a centromere that divides the pair into four arms.

Zheng, who is now an assistant professor of [Bioinformatics and Computational Biology](#) at the University of Texas MD Anderson Cancer Center, found that overproduction of histones is positively correlated with the whole-arm chromosome losses in brain and breast cancer.

The finding is consistent with other research linking the overproduction of histones with breaks to the centromere hinges that are essential to making sure each new cell gets a complete set of chromosomes.

Though histone genes comprise only 1/100,000th of the human genome, the over-expression of this tiny, ancient subset of DNA — all by itself — predicted poor outcomes in brain and breast cancers, making it a potentially powerful new biomarker for disease with the potential to improve diagnosis, prognosis and even new therapies.

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