Researchers uncover human DNA repair by nuclear metamorphosis

April 17 2024, by Erin Howe

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Researchers at the University of Toronto have discovered a DNA repair mechanism that advances understanding of how human cells stay healthy, and which could lead to new treatments for cancer and premature aging.

The study, published in the journal *Nature Structural and Molecular*

Biology, also sheds light on the mechanism of action of some existing chemotherapy drugs.

"We think this research solves the mystery of how DNA double-strand breaks and the nuclear envelope connect for repair in human cells," said Professor Karim Mekhail, co-principal investigator on the study and a professor of laboratory medicine and pathobiology at U of T's Temerty Faculty of Medicine.

"It also makes many previously published discoveries in other organisms applicable in the context of human DNA repair, which should help science move even faster."

DNA double-strand breaks arise when cells are exposed to radiation and chemicals, and through internal processes such as DNA replication. They are one of the most serious types of DNA damage because they can stall cell growth or put it in overdrive, promoting aging and cancer.

The new discovery, made in human cells and in collaboration with Professor Razqallah Hakem, a researcher at University Health Network and professor at Temerty Medicine, extends prior research on DNA damage in yeast by Mekhail and other scientists.

In 2015, Mekhail and collaborators showed how motor proteins deep inside the nucleus of yeast cells transport double-strand breaks to "DNA hospital-like" protein complexes embedded in the nuclear envelope at the edge of the nucleus.

Other studies uncovered related mechanisms during DNA repair in flies and other organisms. However, scientists exploring similar mechanisms in human and other mammalian cells reported little to no DNA mobility for most breaks.

"We knew that nuclear envelope proteins were important for DNA repair across most of these organisms, so we wondered how to explain the limited mobility of damaged DNA in mammalian cells," Mekhail says.

The answer is both surprising and elegant.

When DNA inside the nucleus of a human cell is damaged, a specific network of microtubule filaments forms in the cytoplasm around the nucleus and pushes on the nuclear envelope. This prompts the formation of tiny tubes, or tubules, which reach into the nucleus and catch most double-strand breaks.

"It's like fingers pushing on a balloon," says Mekhail. "When you squeeze a balloon, your fingers form tunnels in its structure, which forces some parts of the balloon's exterior inside itself."

Further research by the study authors detailed several aspects of this process. Enzymes called DNA damage response kinases and tubulin acetyltransferase are the master regulators of the process, and promote the formation of the tubules.

Enzymes deposit a chemical mark on a specific part of the microtubule filaments, which causes them to recruit tiny motor proteins and push on the nuclear envelope. Consequently, the repair-promoting protein complexes push the envelope deep into the nucleus, creating bridges to the DNA breaks.

"This ensures that the nucleus undergoes a form of reversible metamorphosis, allowing the envelope to temporarily infiltrate DNA throughout the nucleus, capturing and reconnecting broken DNA," says Mekhail.

The findings have significant implications for some cancer treatments.

Normal cells use the nuclear envelope tubules to repair DNA, but cancer cells appear to need them more. To explore the mechanism's potential impact, the team analyzed data representing over 8,500 patients with various cancers. The need was visible in several cancers, including triple-negative breast cancer, which is highly aggressive.

"There is a huge effort to identify new therapeutic avenues for cancer patients, and this discovery is a big step forward," says Hakem, a senior scientist at UHN's Princess Margaret Cancer Center and a professor in U of T's department of medical biophysics and department of laboratory medicine and pathobiology.

"Until now, scientists were unclear as to the relative impact of the nuclear envelope in the repair of damaged DNA in human cells. Our collaboration revealed that targeting factors that modulate the nuclear envelope for damaged DNA repair effectively restrains breast cancer development," Hakem says.

In the aggressive triple negative breast cancer, there are elevated levels of the tubules, likely because they have more DNA damage than normal cells. When the researchers knocked out the genes needed to control the tubules, cancer cells were less able to form tumors.

One medication used to treat triple negative breast cancer is a class of drugs called PARP inhibitors. PARP is an enzyme that binds to and helps repair damaged DNA. PARP inhibitors block the enzyme from performing repair, preventing the ends of a DNA double-strand break in cancer cells from reconnecting to one another.

The cancer cells end up joining two broken ends that are not part of the same pair. As more mismatched pairs are created, the resulting DNA

structures become impossible for cells to copy and divide.

"Our study shows that the drug's ability to trigger these mismatches relies on the tubules. When fewer tubules are present, cancer cells are more resistant to PARP inhibitors," says Hakem.

Partnerships among researchers in distinct fields was essential for the findings in cancer cells. The study underscores the importance of cross-disciplinary collaboration, Mekhail says.

"The brain power behind every project is crucial. Every team member counts. Also, every right collaborator added to the research project is akin to earning another doctorate in a new specialty; it's powerful," he says.

Mekhail notes the discovery is also relevant to premature aging conditions like progeria. The rare genetic condition causes rapid aging within the first two decades of life, commonly leading to early death.

Progeria is linked to a gene coding for lamin A. Mutations in this gene reduce the rigidity of the nuclear envelope. The team found that expression of mutant lamin A is sufficient to induce the tubules, which DNA damaging agents further boosted. The team thinks that even weak pressure on the nuclear envelope spurs the creation of tubules in premature aging cells.

The findings suggest that in progeria, DNA repair may be compromised by the presence of too many or poorly regulated tubules. The study results also have implications for many other clinical conditions, Mekhail says.

"It's exciting to think about where these findings will lead us next," says Mekhail. "We have excellent colleagues and incredible trainees here at

Temerty Medicine and in our partner hospitals. We're already working toward following this discovery and using our work to create novel therapeutics."

More information: DNA double-strand break–capturing nuclear envelope tubules drive DNA repair, *Nature Structural & Molecular Biology* (2024). DOI: 10.1038/s41594-024-01286-7

Provided by University of Toronto