Where cigarette smoking's damage is done... down to your DNA

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Scientists have known for decades that smoking cigarettes causes DNA damage, which leads to lung cancer. Now, for the first time, UNC School of Medicine scientists created a method for effectively mapping that DNA damage at high resolution across the genome. Credit: Christ-claude Mowandza-ndinga (UNC Health Care)

Scientists have known for decades that smoking cigarettes causes DNA damage, which leads to lung cancer. Now, for the first time, UNC School of Medicine scientists created a method for effectively mapping that DNA damage at high resolution across the genome.

The innovation comes from the laboratory of Nobel laureate Aziz Sancar, MD, PhD, the Sarah Graham Kenan Professor of Biochemistry and Biophysics at UNC's School of Medicine. In a study published in the *Proceedings of the National Academy of Sciences*, Sancar and his team developed a useful technique for mapping sites on the genome that are undergoing repair following a common type of DNA damage. They then used that technique to map all damage caused by the major chemical carcinogen - benzo[α]pyrene.

"This is a carcinogen that accounts for about 30 percent of the cancer deaths in the United States, and we now have a genome-wide map of the damage it causes," Sancar said.

Maps like these will help scientists better understand how smoking-induced cancers originate, why some people are more vulnerable or resistant to cancers, and how these cancers might be prevented. Sancar also hopes that providing such stark and specific evidence of smoking's harm at the cellular level might induce some smokers to kick the habit. There are about 40 million smokers in the United States and a billion worldwide.

"It would be good if this helps raise awareness of how harmful smoking can be," he said. "It also would be helpful to drug developers if we knew exactly how DNA damage is repaired throughout the entire genome."

BaP: Earth's Top Chemical Carcinogen?

Benzo[α]pyrene (BaP) is a member of a family of simple, hardy, carbon-

rich hydrocarbons - polycyclic aromatic hydrocarbons - that can form even in outer space. Scientists think these molecules might have seeded simple carbon-based life on Earth and other planets. But for more evolved and complex DNA-based life forms - humans for example - BaP poses a serious environmental hazard. It's a byproduct of burning organic compounds, such as tobacco plants. Everyday forms of combustion, from forest fires to diesel engines and barbecue grills, put a lot of BaP into our air, soil, and food. But nothing in ordinary life delivers it into human tissue more efficiently than puffing on a lit cigarette.

Typically, when a toxic hydrocarbon gets into a person through breathing or eating, enzymes in our blood break it down into smaller, safer molecules. That happens for BaP, too, but the protective reactions also yield a compound called benzo[α]pyrene diol epoxide (BPDE), which turns out to be worse than BaP itself.

BPDE reacts chemically with DNA, forming a very tight bond at the nucleobase guanine. This bond, or adduct, means that the genes can no longer make proper proteins and DNA can't be duplicated properly during cell division. And if that happens, disease can be the result.

"If a BPDE adduct occurs in a tumor suppressor gene and isn't repaired in a timely manner, it can lead to a permanent mutation that turns a cell cancerous," said Wentao Li, PhD, a postdoctoral researcher and lead author of the study.

There is no doubt about the basic carcinogenicity of chemical reaction. Paint a moderate dose of BaP on the skin of a lab mouse, and tumors are almost certain to erupt. BaP, via BPDE, has long been recognized as a promoter of multiple types of cancer and is considered the single most important cause of lung cancer.

Repairs underway

Sancar's new method for mapping BaP-induced DNA damage enables scientists to identify the sites on the genome where cells are trying to repair the damage. Sancar won a share of the 2015 Nobel Prize for Chemistry for teasing apart the detailed workings of this biochemical repair process.

Known as nucleotide excision repair, it involves the recruitment of special proteins that perform DNA surgery. They snip out the affected strand of DNA. If all goes well, DNA-synthesizing enzymes then reconstruct the missing section of DNA from another unaffected strand. This is possible because all cell-based life forms on Earth have two complementary strands of DNA. Meanwhile, the snipped-out damaged section of DNA floats free until garbage-disposal molecules eventually degrade it.

Those free-floating bits of damaged DNA may be garbage to the cell, but they are solid gold for a scientist who wants to map all damage in a genome. With the new method, scientists can tag and collect these cast-off snippets, sequence them, and then fit together their sequences - like tiny pieces of a giant puzzle - to create a map of the genome. In the end, scientists have a complete map of the sites where repairs to damaged DNA have begun.

Given the effort and expense required for DNA sequencing, the initial, proof-of-principle map published by Sancar, Li and colleagues doesn't have the highest resolution possible. But it points the way towards the routine scientific use of such maps, especially as costs drop, to better understand how DNA-damaging events lead to disease and death.

This mapping technique should help answer several questions, such as:

- What dose of a toxin is needed to overwhelm the average person's nucleotide excision repair capacity?

- Which variations - and in which genes - give people more or less capacity to repair such DNA damage?
- Are there certain spots on the genome where successful repairs are inherently less likely?

Even with their initial, medium-resolution map, Sancar and colleagues were able to show that repairs of BPDE damage tend to occur more often when the BPDE-burdened guanine (G) is next to a cytosine (C) rather than a thymine (T) or adenine (A). This suggests there are "hotspots" of higher risk for BPDE-induced mutation.

"Understanding this bias in repair should help us better understand why exposures to toxins such as BaP tend to cause certain gene mutations," Li said.

Looking forward

In studies published in 2015 and 2016, Sancar and colleagues used earlier versions of their technique to map two other types of DNA-adduct damage: one wrought by ultraviolet light and the other by the common chemo drug cisplatin. Those mapping studies required an extra chemical step - removing the damage from an excised snippet before sequencing it - because the DNA-reading enzyme needed for the sequencing process would otherwise get stuck at the adduct. In contrast, the new technique employs "translesional" enzymes with dimensions that allow it to keep reading a strand of DNA even when a bulky BPDE adduct is present.

"This new method can be applied to any type of DNA damage that involves nucleotide excision repair," Sancar said.

Sancar, Li, and their colleagues are now using the new technique to map DNA damage repair associated with other environmental toxins. Their

next project focuses on aflatoxins, a family of mold-produced molecules often found in poorly stored nuts and grains. These toxins damage DNA and are major causes of liver cancer in developing countries.

The researchers are also performing more studies to uncover factors influencing where and whether nucleotide excision DNA repair occurs. To do that, they need to map sites of actual damage on the genome itself, not just the damaged snippets that are excised during repairs.

In one such project, they have developed a sensitive, high-resolution method for mapping actual DNA damage caused by ultraviolet light. By combining that method with repair mapping, they have found that the UV damage to DNA appears to be essentially uniform, although the repair process is not. Repair seems to be affected by a host of factors, including how actively a given stretch of DNA is being copied out to encode the making of proteins. They are currently applying this method to BaP to complement the repair map they have generated.

That again points to the likelihood of hotspots where repair is less likely to occur and mutations are more likely to arise.

"I'm certain," said Sancar, "that all this information will lead to a better understanding of why certain people are predisposed to cancer, and which smoking-related mutations lead to lung cancer specifically."

And that, in turn, could have implications for the development of more targeting therapies down the line.

More information: Wentao Li el al., "Human genome-wide repair map of DNA damage caused by the cigarette smoke carcinogen benzo[a]pyrene," *PNAS* (2017). www.pnas.org/cgi/doi/10.1073/pnas.1706021114

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