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Customized DNA rings aid early cancer detection in mice, study finds

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Imagine: You pop a pill into your mouth and swallow it. It dissolves, releasing tiny particles that are absorbed and cause only cancerous cells to secrete a specific protein into your bloodstream. Two days from now, a finger-prick blood sample will expose whether you've got cancer and even give a rough idea of its extent.

That's a highly futuristic concept. But its realization may be only years, not decades, away.

Stanford University School of Medicine investigators administered a customized genetic construct consisting of tiny rings of DNA, called DNA minicircles, to mice. The scientists then showed that mice with tumors produced a substance that tumor-free mice didn't make. The substance was easily detected 48 hours later by a simple blood test.

A paper describing the findings of this proof-of-principle study will be published online Feb. 23 in the *Proceedings of the National Academy of Sciences*.

The technique has the potential to apply to a broad range of cancers, so someday clinicians might be able not only to detect tumors, monitor the effectiveness of cancer therapies and guide the developments of anti-tumor drugs, but—importantly—to screen symptom-free populations for nascent tumors that might have otherwise gone undetected until they became larger and much tougher to treat.

Triggering an unambiguous biomarker

The hunt for cancer biomarkers—substances whose presence in an individual's blood or urine flags a probable tumor—is nothing new, said the study's senior author, Sanjiv "Sam" Gambhir, professor and chair of radiology and director of the Canary Center at Stanford for Cancer Early Detection. High blood levels of prostate-specific antigen, for example, can signify prostate cancer, and there are also biomarkers that sometimes signal ovarian and colorectal cancer, he said.

But while various tumor types naturally secrete characteristic substances into the blood, the secreted substance is typically specific to the tumor type, with each requiring its own separate test. Complicating matters, these substances are also quite often made in healthy tissues, so a positive test result doesn't absolutely mean a person actually has cancer. Or a tumor—especially a small one—simply may not secrete enough of the trademark substance to be detectable.

Gambhir's team appears to have found a way to force any of numerous tumor types to produce a biomarker whose presence in the blood of mice unambiguously signifies cancer, because none of the rodents' tissues—cancerous or otherwise—would normally be making it.

This biomarker is a protein called secreted embryonic alkaline phosphatase. SEAP is naturally produced in human embryos as they form and develop, but it's not present in adults.

Tricking cancer cells

To trick mice's cancer cells into making SEAP and squirting it into the bloodstream, Gambhir and his colleagues used a DNA minicircle: a tiny, artificial, single-stranded DNA ring about 4,000 nucleotides in

circumference—or roughly one-millionth as long as the DNA strand that would result from stretching all 23 chromosomes of the human genome end to end.

The minicircles, which contained a single gene coding for SEAP, were mixed with a chemical agent to facilitate their uptake by both healthy and cancerous cells alike.

In front of the gene, the researchers inserted a short DNA sequence called a promoter. Promoters don't code for protein production but, rather, are more like switches or rheostats telling the cell's protein-manufacturing machinery whether and when to actually make the protein the gene codes for, and how much. Genes in every cell of all multicelled creatures are preceded by promoters. Although all of an individual's cells contain precisely the same genes, how "on" the promoter is for a given gene in a given cell depends on varying local conditions within that particular cell at that particular time.

The particular promoter Gambhir and his colleagues snapped into the minicircle ahead of the SEAP gene normally regulates a gene called survivin that, in adults, is only "on" in cancer cells. So, in theory, the SEAP gene on the DNA minicircles Gambhir's lab created would be produced only in cancer cells.

The survivin promoter has been used in gene-therapy experiments and is well-studied, Gambhir said. He noted that the survivin promoter is activated in a broad range of cancers, including breast, lung, ovarian and other major tumor types.

For the study, the researchers injected cells from human melanoma cell lines into laboratory mice. Ordinarily, a mouse's immune system would attack any injected human cell, cancerous or not. But these mice were immune-compromised, and soon numerous tiny tumors started to

develop throughout their bodies, especially in their lungs. Other mice of the same strain were given otherwise-identical injections that didn't contain the cancer cells; they didn't develop tumors.

About two weeks later, the animals' blood was checked to make sure their baseline levels of SEAP were zero. Then the investigators injected the minicircles intravenously into the animals' tail veins and measured SEAP levels in the mice's blood one, three, seven, 11 and 14 days later. Within 48 hours, SEAP was present in the blood of mice with tumors, but not in that of the tumor-free animals. That signal began declining in strength as early as 72 hours post-injection, fading to insignificance within the next two weeks or so. Its maximum strength varied with the total tumor volume in a mouse's lungs, suggesting that the test may be sensitive not only to the presence of cancer but also to its extent.

Advantages of minicircles

DNA minicircles carry advantages over familiar gene-therapy workhorses such as defanged viruses or naturally occurring rings of DNA called plasmids, which bacteria trade back and forth along with the antibiotic-resistance genes plasmids often contain. DNA minicircles lack the apparatus for replicating themselves inside of a cell. Importantly, they're also incapable of integrating randomly into a host cell's genome—and, possibly, right into the middle of a gene, disrupting that gene's operation. Nor do they contain stretches of bacterial DNA that could elicit a strong immune response in people, as viruses and plasmids do.

"We want to translate this strategy into humans, so we've set it up in a way that's most likely to be effective, safe and convenient," Gambhir said. Although the minicircles were injected intravenously to the mice in this study, it should eventually prove possible to deliver them orally via a pill, he said. "We haven't got it down to a pill yet, but the oral delivery

part of this is likely a solvable problem—only a few years off, not five or 10 years off."

It will take much more time than that to prove that the approach is safe to use in humans, though, Gambhir cautioned. Eventually, he said, he foresees variations, such as adding a second gene to the minicircles. For example, if minicircles incorporated a gene coding for a protein that stays inside a cancer cell instead of being secreted and can be imaged remotely, they could enable not only the detection of a small tumor hidden inside an organ such as the liver, lung or pancreas, but the pinpointing of its location. A gene for treating cancer could also be added to the minicircles, allowing physicians to use them for both diagnosis and therapy.

More information: *PNAS*
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