Researchers silence leading cancer-causing gene

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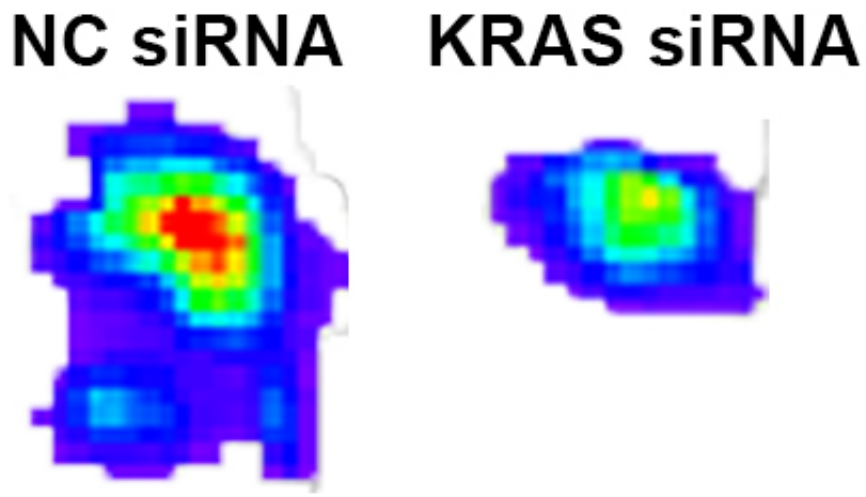

Using bioluminescence, researchers showed that the novel molecule 'KRAS silencing RNA' or 'KRAS siRNA' (right) reduced the size of a tumor in mice. Researchers used a 'non-KRAS silencing' molecule as the control (left) as a comparison. Credit: Chad Pecot, MD, UNC Lineberger Comprehensive Cancer Center

Researchers from the UNC School of Medicine and colleagues at The University of Texas MD Anderson Cancer Center have developed a new approach to block the KRAS oncogene, one of the most frequently mutated genes in human cancer. The approach, led by Chad Pecot, MD, an assistant professor of medicine at UNC, offers another route to attack KRAS, which has proven to be an elusive and frustrating target for drug

developers.

The new method relies upon a specifically sequenced type of small interfering RNA - or siRNA. The findings, published in the journal *Molecular Cancer Therapeutics*, show that using a form of siRNA to halt KRAS not only dramatically stunted the growth of lung and colon cancers in cultured cells and mice but also stopped metastasis - the main cause of cancer deaths.

"KRAS has been widely regarded as an undruggable protein, but we show that that's simply not the case," said Pecot, the study lead author and member of the UNC Lineberger Comprehensive Cancer Center.

KRAS is a signaling molecule - a protein switch that triggers a cascade of molecular events that tell cells to grow and survive. Mutations in the KRAS gene create a switch that is perpetually "on," causing cells to divide uncontrollably. KRAS mutations are present in roughly 30 percent of human cancers, particularly lung, colon, pancreatic, and thyroid.

"It is the elephant in the room," Pecot said. "KRAS was one of the first cancer-causing genes ever discovered, and it was the obvious target to go after. People have been trying for decades to hit it, but they haven't had much luck."

Inhibiting KRAS signaling has been tricky because it lacks good pockets or crevices for small molecules and drugs to bind to. Some researchers have tried instead to target the proteins downstream in the KRAS signaling cascade, but those attempts have also had limited success.

Rather than try another conventional approach, Pecot decided to use a new genetic tool known as RNA interference - or RNAi - to destroy the KRAS protein before it fully forms. RNAi uses bits of synthetically engineered RNA - the single-stranded molecule transcribed from DNA -

to silence specific genes. These bits of RNA bind to specific genetic messages called mRNA in the cell and direct enzymes to recognize the messages as enemies. In this context, the enzymes destroyed the genetic messages of KRAS mRNA so that KRAS can't be made. As a result, the cells don't grow, replicate, or move nearly as well.

RNAi has shown great promise in the treatment of liver diseases, viral infections, and cancers. To see if this approach could thwart the KRAS oncogene, Pecot and his colleagues first had to test different sequences of RNA to determine which one most effectively tagged KRAS for destruction. Of five RNA sequences, the researchers identified two candidates worthy to take into cancer models.

When they delivered these sequences into tissue culture cells, they found that the siRNAs destroyed more than 90 percent of the KRAS gene messages, significantly impairing the growth of cancer cell lines. The technique also led to marked reduction of two signaling molecules called pERK and pMEK, which lie downstream of KRAS and have been implicated in cancer cell proliferation and tumor growth.

Next, Pecot and his colleagues tested the siRNAs in mouse models of lung and colon cancer. They wrapped the sequences in protective lipid nanoparticles and delivered the siRNA solution into the mice. The researchers found that this treatment significantly slowed the growth of primary tumors. For example, tumors from colon cancer models that had been treated with the KRAS siRNAs were 69 percent smaller than tumors treated with control RNA sequences.

In addition, the researchers discovered that silencing KRAS stemmed the spread of cancer cells to other organs. The siRNA treatment reduced the number of these secondary malignant growths by about 80 percent in mice with lung cancer and to a similar degree in colon cancer models.

Pecot's findings come on the heels of two other papers using siRNAs to target KRAS, one from Frank McCormick's laboratory at the University of California at San Francisco and the other from Tyler Jacks' laboratory at the Massachusetts Institute of Technology. What sets the UNC study apart is that it demonstrates that this approach can be used to control the development of metastatic disease.

"Having all three papers come out at about the same time is encouraging because it means that KRAS is druggable if you use outside-the-box methods," Pecot said. "Now, we essentially have three platforms for targeting KRAS with siRNAs that may get to the clinic."

Pecot said the results, while promising, are just a first step in combating this cancer-causing gene. Ultimately, the siRNA sequences will have to be designed to specifically target the mutant form of KRAS without disrupting the normal form of the gene, which is necessary for maintaining normal growth in healthy cells.

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