Researchers provide proof in humans of RNA interference using targeted nanoparticles

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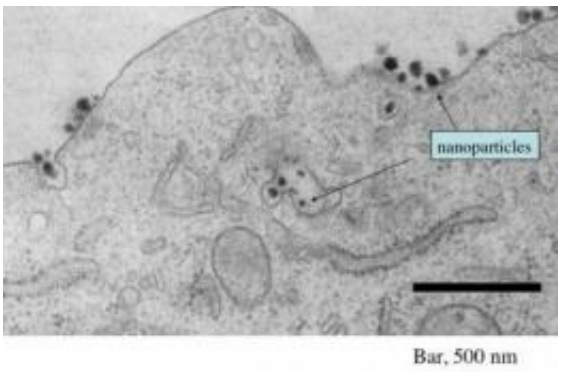

This electron micrograph shows the presence of numerous siRNA-containing targeted nanoparticles both entering and within a tumor cell. Credit: Caltech/Swaroop Mishra

A California Institute of Technology (Caltech)-led team of researchers and clinicians has published the first proof that a targeted nanoparticle -- used as an experimental therapeutic and injected directly into a patient's bloodstream -- can traffic into tumors, deliver double-stranded small interfering RNAs (siRNAs), and turn off an important cancer gene using a mechanism known as RNA interference (RNAi). Moreover, the team provided the first demonstration that this new type of therapy, infused into the bloodstream, can make its way to human tumors in a dose-dependent fashion -- i.e., a higher number of nanoparticles sent into the

body leads to a higher number of nanoparticles in the tumor cells.

These results, published in the March 21 advance online edition of the journal *Nature*, demonstrate the feasibility of using both nanoparticles and RNAi-based therapeutics in patients, and open the door for future "game-changing" therapeutics that attack cancer and other diseases at the genetic level, says Mark Davis, the Warren and Katharine Schlinger Professor of Chemical Engineering at Caltech, and the research team's leader.

The discovery of RNA interference, the mechanism by which double strands of RNA silence genes, won researchers Andrew Fire and Craig Mello the 2006 Nobel Prize in Physiology or Medicine. The scientists first reported finding this novel mechanism in worms in a 1998 *Nature* paper. Since then, the potential for this type of gene inhibition to lead to new therapies for diseases like cancer has been highly touted.

"RNAi is a new way to stop the production of proteins," says Davis. What makes it such a potentially powerful tool, he adds, is the fact that its target is not a protein. The vulnerable areas of a protein may be hidden within its three-dimensional folds, making it difficult for many therapeutics to reach them. In contrast, RNA interference targets the messenger RNA (mRNA) that encodes the information needed to make a protein in the first place.

"In principle," says Davis, "that means every protein now is druggable because its inhibition is accomplished by destroying the mRNA. And we can go after mRNAs in a very designed way given all the genomic data that are and will become available."

Still, there have been numerous potential roadblocks to the application of RNAi technology as therapy in humans. One of the most problematic has been finding a way to ferry the therapeutics, which are made up of

fragile siRNAs, into tumor cells after direct injection into the bloodstream. Davis, however, had a solution. Even before the discovery of RNAi, he and his team had begun working on ways to deliver nucleic acids into cells via systemic administration. They eventually created a four-component system—featuring a unique polymer—that can self-assemble into a targeted, siRNA-containing nanoparticle. The siRNA delivery system is under clinical development by Calando Pharmaceuticals, Inc., a Pasadena-based nanobiotech company.

"These nanoparticles are able to take the siRNAs to the targeted site within the body," says Davis. Once they reach their target—in this case, the cancer cells within tumors—the nanoparticles enter the cells and release the siRNAs.

The scientific results described in the *Nature* paper are from a Phase I clinical trial of these nanoparticles that began treating patients in May 2008. Phase I trials are, by definition, safety trials; the idea is to see if and at what level the drug or other therapy turns harmful or toxic. These trials can also provide an in-human scientific proof of concept—which is exactly what is being reported in the Nature paper.

This targeted nanoparticle used in the study and shown in this schematic is made of a unique polymer and can make its way to human tumor cells in a dose-

dependent fashion. Credit: Caltech/Derek Bartlett

Using a new technique developed at Caltech, the team was able to detect and image nanoparticles inside cells biopsied from the tumors of several of the trial's participants. In addition, Davis and his colleagues were able to show that the higher the nanoparticle dose administered to the patient, the higher the number of particles found inside the tumor cells—the first example of this kind of dose-dependent response using targeted nanoparticles.

Even better, Davis says, the evidence showed the siRNAs had done their job. In the tumor cells analyzed by the researchers, the mRNA encoding the cell-growth protein ribonucleotide reductase had been degraded. This degradation, in turn, led to a loss of the protein.

More to the point, the mRNA fragments found were exactly the length and sequence they should be if they'd been cleaved in the spot targeted by the siRNA, notes Davis. "It's the first time anyone has found an RNA fragment from a patient's cells showing the mRNA was cut at exactly the right base via the RNAi mechanism," he says. "It proves that the RNAi mechanism can happen using siRNA in a human."

"There are many cancer targets that can be efficiently blocked in the laboratory using siRNA, but blocking them in the clinic has been elusive," says Antoni Ribas, associate professor of medicine and surgery at UCLA's Jonsson Comprehensive Cancer Center. "This is because many of these targets are not amenable to be blocked by traditionally designed anti-cancer drugs. This research provides the first evidence that what works in the lab could help patients in the future by the specific delivery of siRNA using targeted nanoparticles. We can start thinking about targeting the untargetable."

"Although these data are very early and more research is needed, this is a promising study of a novel cancer agent, and we are proud of our contribution to the initial clinical development of siRNA for the treatment of cancer," says Anthony Tolcher, director of clinical research at South Texas Accelerated Research Therapeutics (START).

"Promising data from the clinical trials validates our years of research at City of Hope into ribonucleotide reductase as a target for novel gene-based therapies for cancer," adds coauthor Yun Yen, associate director for translational research at City of Hope. "We are seeing for the first time the utility of siRNA as a cancer therapy and how nanotechnology can target cancer cells specifically."

The Phase I trial—sponsored by Calando Pharmaceuticals—is proceeding at START and UCLA's Jonsson Comprehensive Cancer Center, and the clinical results of the trial will be presented at a later time. "At the very least, we've proven that the RNAi mechanism can be used in humans for therapy and that the targeted delivery of siRNA allows for systemic administration," Davis says. "It is a very exciting time."

More information: "Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles," *Natre*, March 22, 2010.

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