First evidence of RNA interference mechanism induced in human
Getting closer to a cure?

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Knocking down a human gene by RNA silencing has been the focus of a number of laboratories since the discovery of the RNAi mechanism more than ten years ago. In their remarkable work, M. Davis and collaborators show for the first time the induction of an RNAi pathway in humans following systemic administration of nanoparticles.2 In a clinical trial, they demonstrate the accumulation of the particles in the tumor, as well as the downregulation of mRNA and protein expression of the target gene. Moreover, they successfully linked these observations to the induction of mRNA cleavage by the delivered siRNA in the tumor.

Introduction

The first observation of an RNA silencing phenomenon in plants in the early 1990s opened an extraordinary field of possibilities and led to extensive search for similar mechanisms in other organisms. The decisive work of Fire and Mello, awarded the Nobel Prize in Physiology or Medicine in 2006, demonstrated a potent gene silencing effect after injecting double stranded RNA in C. elegans.1 Using RNA interference (RNAi) technology to knockdown a gene and cure diseases (among them cancer) has seemed promising ever since. However, it has been challenged by the difficulty in delivering the siRNA to the target tissue (siRNA stability, toxicity, immunogenicity, targeting).

In their pioneer work published earlier this year in Nature, M. Davis and collaborators evidenced for the first time the induction of an RNAi mechanism in humans by systemic administration of targeted nanoparticles.2

Cyclodextrin-Based Nanoparticles Systemically Administered to Solid Tumors

Since 1999 and its first publication on cyclodextrin-based macromolecular therapeutic delivery,3 M. Davis’s laboratory has provided extensive studies on the chemistry, toxicity, as well as on the in vitro and in vivo effects of the particles used in the present work. These synthetic self-assembled particles are composed of four different elements conferring multivalency, stability, targeting and silencing to the system.

The siRNA is designed to silence the M2 subunit of ribonucleotide reductase (RRM2), an established anti-cancer target. It is complexed with a linear cyclodextrin-based polymer. The cyclodextrin is itself linked via adamantane to a hydrophilic polymer (polyethylene glycol, PEG) on which the human transferrin ligand is attached. Quiescent cells (e.g., malignant cells) express a high level of transferrin receptor. The ligand is used to engage the receptor on the surface of cancer cells, favoring cellular uptake of the particles. The PEG linker helps to stabilize the system in biological fluids.

The clinical trial included three patients with solid melanoma cancers refractory to standard-of-care therapy. The treatment was administered by a 30 min intravenous infusion on days 1, 3, 8 and 10 of a 21-day cycle. Three different doses of siRNA were tested: patient A was infused 18 mgm-2 per dose, patient B, 24 mgm-2 and patient C, 30 mgm-2. Patient C went on a second cycle of siRNA administration, which started around 1 month after the end of cycle 1.
The effects of particles delivery were characterized on biopsy samples taken after the final dose of cycle 1 and compared to archived tissues. Patient C’s final biopsy is from the day of the last cycle 2 injection.

**Dose-Dependent Delivery of the Nanoparticles**

The biopsies were studied for nanoparticles localization and distribution.

Transmission electron microscopy analyses established the intracellular localization of the particles. The siRNA, linked to the fluorochrome Cy3, was directly visualized by fluorescence microscopy. To detect the delivery system, the authors used 5 nm gold particles linked via adamantane-PEG to the cyclodextrin polymers. The method was first confirmed both in vitro and in vivo.

The gold staining revealed that the nanoparticles were heterogeneously distributed in biopsies taken at the end of cycle 1, whereas all three archived biopsies were free of nanoparticles. The staining was high (both in intensity and number of regions stained) in patient C (receiving the highest dose of siRNA), intermediate in patient B and not observed in patient A (with the lowest dose of siRNA). After the second cycle of siRNA administration, biopsy of patient C confirmed a high level of nanoparticles compared to patient A and B (cycle 1).

These observations constitute the first demonstration of dose-dependent accumulation of nanoparticles of any kind in humans.

**Knockdown of the Target Protein**

Quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR), immunohistochemistry (IHC) and western blots established that both RRM2 mRNA and protein expression were decreased after administration of the nanoparticles.

While the decrease of RRM2 mRNA in patients A and B could not be directly attributed to the siRNA administration because the archived tissues were taken long before the trial, patient C clearly exhibited a decrease of RRM2 protein during the time of the trial (quantified by IHC).

Moreover, after the second cycle, both mRNA and protein in patient C were reduced when compared to the biopsy taken at the beginning of the second cycle (ten days between the two biopsies).

It is worth noting that the IHC analyses from patient A and B did not show a clear decrease of the protein expressed in the tumor, underlining the causality between the dose administered and the observed effect.

**Induction of an RNAi Mechanism**

The nanoparticles developed by M. Davis and collaborators were delivered to the tumor and a decrease of both RRM2 mRNA and protein was observed after siRNA administration. To formally link the knockdown of the gene to an RNA interference pathway, the authors used a molecular method: a modified 5’-RNA-ligand-mediated RACE (rapid amplification of complementary DNA ends). This technique allows the characterization of defined mRNA cleavage products.

RRM2 mRNA 5’ fragment was detected in patient C’s samples taken at the beginning and at the end of the second cycle of siRNA administration. This fragment was not detected in patients A and B. These results demonstrate for the first time in humans that siRNA systematically administered can induce the RNAi machinery in solid tumors and cause the expected mRNA cleavage.

Moreover, the cleavage product of RRM2 mRNA was detected before the start of the second cycle, showing the effect of the first cycle of siRNA administration. Hence, the delivery of these nanoparticles engaged the RNAi pathway for several weeks.

**Conclusion**

This work constitutes the first demonstration of an RNAi mechanism engaged in humans after systemic administration of nanoparticles. Such a result not only justifies the sustained work of many laboratories around the world, but also gives great hopes regarding the feasibility of future siRNA therapies.

However, further studies are required. Indeed, the demonstration of the RRM2 protein knockdown and of the RNAi pathway has been formally established for only one patient. Moreover, this patient had followed two cycles of drug administration at the highest dose tested. The few samples available did not allow to link with certainty the decrease of mRNA observed in patient A and B to the treatment, or to study the progression in patient C from the first cycle of siRNA administration.

RNA silencing was induced by the administration of self-assembled synthetic nanoparticles, for which more than 20 publications by the same group have previously been reported. Chemistry, as well as biological implications (toxicity, immunogenicity) have been extensively studied and result in a “solid” system. A direct correlation between the dose administered and the accumulation of the particles in the tumor has been shown for the first time.

Despite this success, there is room for substantial improvements and open questions remain. For instance, one important point for further clinical trials is a better understanding of the particles dissociation kinetic. Indeed, because the nanoparticles are not observed at the beginning of the second cycle of treatment in patient C, they must disassemble within a month. Knowing how long the particles really reside within the cells and release siRNA would allow refinement of the administration protocol.

This system could also be improved by taking advantage of the targeting. As M. Davis and collaborators reported previously, the transferrin ligand does not affect the biodistribution of the particle; it mainly improves its intracellular uptake.

The penetration of the drug could be further increased if coupled to a tumor penetrating peptide with targeting capacities. In a recently published work, Sugahara and collaborators described such a peptide, iRGD, which presents the particularity to not only promote active targeting to the tumor, but to also enhance vascular and tissue permeability.

Co-administered with various drugs, among them nanoparticles (nab-paclitaxel and doxorubicin liposomes), it promotes their penetration.
into the tumor. One can envision that co-
administration of this peptide with the
cyclodextrin-siRNA complex developed
by M. Davis’s group would enhance the
efficacy of the drug while reducing its side-
effects, getting a step closer to the cure.

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