Plan B for anti-miRNA

By Lev Osherovich, Senior Writer

Formulating and delivering therapeutic anti-microRNAs has been a big challenge in the burgeoning anti-miRNA space. Now, a **University of California, San Diego** team has proof of concept for a surprisingly simple way to deliver these molecules into the bloodstream using engineered B cells.¹

The UCSD group, led by Professor of Medicine Maurizio Zanetti, used murine B cells transfected with an RNA-encoding construct to knock down expression of an endogenous miRNA in T cells, which work closely with B cells during immune activity (see Figure 1, “Anti-miRNA delivery by B cells”).

The new delivery technique exploits exosomes—small, lipid-bound vesicles that are naturally secreted by a variety of cells—to transfer the anti-miRNA from the engineered B cells to the target cells.

“The novelty is that you can use a cell that is particularly abundant in the blood to deliver a customized immunogenomic treatment,” said Zanetti. “You can imagine exploiting this property to attack different disease processes, be it cancer, immune response or inflammation.”

Determining whether the technique can target RNA in other cells besides T cells and whether the magnitude of the knockdown is enough for a therapeutic effect will require further preclinical work.

Exosome relay

Zanetti’s team started by harvesting B cells from mice and transfecting them with a plasmid encoding a 22-base-pair sequence that was complementary and thus antagonistic to miR-150. miR-150 influences both B and T cell differentiation.

In cell culture, transfected B cells produced large amounts of anti-miR-150 RNA and released the transcript into the medium. The RNA was primarily encapsulated in exosomes secreted by the B cells.

Figure 1. Anti-miRNA delivery by B cells. Almanza *et al.* have engineered B cells to deliver anti-microRNA to other immune cells. The findings suggest a new avenue for delivering therapeutic miRNA.

A plasmid (a) engineered to encode a short sequence complementary to microRNA-150 (anti-miR-150) is transfected in murine B cells (b). In cell culture and *in vivo*, engineered B cells interact with T cells, which naturally express miR-150 (c). When B cells and T cells interact to form an immunological synapse (d), anti-miR-150 is secreted into membrane-bound extracellular vesicles (exosomes), which are absorbed by the T cells (e). This leads to degradation and knockdown of endogenous miR-150 (f).

Next, the group simulated conditions that activate T cells *in vitro* by coculturing dendritic cells and T cells in media previously used to grow the anti-miR-150-transfected B cells.

In the presence of a peptide antigen, T cells became activated and absorbed B cell-derived exosomes. As a result, anti-miR-150 penetrated into the T cells and knocked down expression of *miR-150* by about 70%, whereas mock-treated control cells showed no knockdown.

Similar results occurred *in vivo*. T cells from the spleens of mice that received anti-miR-150-transfected B cells had about 60% lower miR-150 than control cells.

Results were reported in the *Proceedings of the National Academy of Sciences*.

Special delivery

Zanetti’s findings demonstrate the feasibility of using B cells to manufacture bioavailable anti-miRNAs. However, many questions

remain about whether the technique would be effective beyond the specific scenario of T cell activation.

Neil Gibson, CSO of miRNA company **Regulus Therapeutics Inc.**, said that the principal novelty of Zanetti's study is that transfected B cells can produce functional anti-miRNAs.

Most techniques in clinical development for knocking down RNA rely on synthetic lipid nanoparticles or chemical conjugates to shield the therapeutic RNA molecules from degradation or excretion. There are no disclosed RNA-targeted therapeutic candidates in the clinic that specifically target B or T cells.

"We had in general thought you couldn't deliver to B cells, though nanoparticle and conjugate strategies have been tried," said Gibson. "But this is taking another step where you use the B cells almost as manufacturing machines for nucleic acid therapeutics."

Gibson said that the study suggests exosomes "are the critical mechanism for transfer of anti-miRNAs. This raises the possibility that if you have a cell with a high capacity for generating exosomes, this could be a way to deliver anti-miRNAs to other cell types that are in close proximity."

Other cell types could include a variety of T cells, innate immune cells and possibly cancer cells.

Regulus' most advanced candidate is RG-101, an anti-miRNA therapeutic that will enter Phase I testing for HCV in 2014.

Zanetti thinks that engineered B cells could be used as autologous cell therapy for a range of autoimmune, inflammatory and cancer indications.

"B cells have the capacity to internalize plasmid or bacterial DNA and are relatively abundant—about 15% of cells in blood—so you don't have to scale them up," said Zanetti.

It is unclear how long B cell-derived exosomes and their anti-miRNA cargo will persist *in vivo* and whether other cell types besides T cells can take them up. Details on the *in vivo* properties of anti-miR-150 were not reported in the *PNAS* paper.

"They show that the B cells are producing anti-miRNA, but will they get enough secretion to do something useful?" asked Matthew Scholz, founder and CEO of **Immusoft Corp.**

Immusoft is developing a platform for *in vivo*, B cell-based manufacturing of antiviral mAbs and enzyme-replacement therapies.

"B cells can persist and produce their payload for a few weeks," said Scholz, but whether the amount of anti-miRNA produced by Zanetti's

transfected B cells is an effective dose in actual disease needs to be determined.

Scholz also wanted to see side-by-side comparisons of anti-miRNAs from engineered B cells with current synthetic lipid formulations "to see if the exosomes secreted naturally are equivalent to some of the synthetic liposomes."

He noted that pharmacodynamic data about Zanetti's anti-miRNAs could make or break the case for using B cell-derived RNA over conventional *in vitro* formulations. If the pharmacodynamics of the two types of anti-miRNA are comparable, Scholz suspects that "the B cells may not even be necessary."

Gibson said that Zanetti's next step should be to test the engineered B cells in a disease model. He suggested inflammatory or autoimmune indications such as rheumatoid arthritis (RA), in which dysregulated T cells play a critical role.

"There are clearly opportunities in the immune modulation space to see whether you can sensitize the immune system" with anti-miRNAs, said Gibson.

Zanetti agreed, adding that B cell-derived anti-miRNAs could also be useful in cancer immunotherapy provided that the B cells can be locally delivered to the tumor site.

"The regulation of the immune system such as proposed in the paper is one therapeutic possibility," he said. "The other is to target cancer tissues and solid tumors, but you would have to improve the targeting of B cells to tumors."

The **University of California** has filed patents on the technique, and the IP is available for licensing.

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