ModeRNA approach to iPS cells

Tracey Baas, Senior Editor

A Boston-based team has used synthetic, modified mRNAs to revert somatic cells to pluripotency, thus taking retroviral vectors out of the reprogramming equation.[1] As an added bonus, the modified-mRNA protocol worked in about half the time of the retroviral protocol and led to 36-fold higher conversion efficiency.

ModeRNA Therapeutics has been founded to commercialize the modified mRNA technology.

The first somatic cell–based production of induced pluripotent stem (iPS) cells was achieved in 2006 by Shinya Yamanaka and colleagues using retroviral vector expression of four transcription factors.[2] The sticking point for this method of reprogramming has been that viral vectors can result in genomic insertions.

Thus, many researchers have sought to deliver the reprogramming factors via alternative routes, including excisable lentiviral or transposon vectors, transient plasmids, non-integrating episomal vectors, RNA-based adenovirus vectors or serial protein transduction.

Those methods all rely on repeat administration of transient vectors, whether DNA or protein based, and typically have low iPS cell yields. Moreover, the recombinant proteins needed for serial delivery can be hard to generate and purify in the quantities required.

A Boston team, led by Derrick Rossi, an assistant professor of pathology at **Harvard Medical School**, decided to take a third path: serial delivery with mRNA. The group produced the mRNAs using standard production techniques but included modified nucleotides and a 5′ guanine cap to boost efficient mRNA translation and half-life. The inclusions also helped avoid cells' antiviral defense systems, which typically target mRNA through interferon- and NF-κB–dependent pathways.

The cap is critical for recognition by the ribosome to start translation and helps protect the mRNA from RNAases.

The researchers then reprogrammed human fibroblasts using serial delivery of the synthetic, modified mRNAs encoding four standard reprogramming transcription factors to produce iPS cells more quickly and efficiently than four retroviruses expressing the same transcription factors.

Gene expression profiling showed that the signatures of iPS cells produced with synthetic, modified mRNAs were more similar to that of human embryonic stem cells (hESCs)—the gold standard of pluripotency—than the signatures of iPS cells produced with retroviruses.

Those findings suggest that RNA-derived iPS cells more fully recapitulate the molecular signature of hESCs and thus may produce higher-quality iPS cells that generate terminally differentiated somatic cells.

Next, the group used modified mRNA encoding the myogenic transcription factor myogenic differentiation 1 (MYOD1; MYOD) to differentiate the newly produced iPS cells to myotubes, which are developing muscle fibers.

"Our work with MYOD was simply a proof-of-concept demonstration that iPS cells could be directed to terminally differentiated fates using our technology," Rossi told *SciBX*.

The findings were described in *Cell Stem Cell*.

This summer, Rossi, along with Kenneth Chien of **Massachusetts General Hospital** and Robert Langer of the **Massachusetts Institute of Technology**, founded ModeRNA to commercialize the findings. The company has backing from Flagship Venture Labs, an arm of Flagship Ventures.

Rossi said that in addition to iPS cells for cell-based therapies and regenerative medicine, the approach may have potential in gene therapy, which also relies on viruses to deliver treatment. He declined to speak further about the company or possible gene-therapy applications.

> **"The standard method to generate iPS cells for clinical applications has yet to be established. I think this method has the potential for it."**
>
> ***—Shinya Yamanaka, University of California, San Francisco***

Cell side analysis

Researchers contacted by *SciBX* agreed that modified mRNAs are a clear addition to the stem cell toolbox but wanted to see a host of additional preclinical studies to further flesh out the properties of the iPS cells.

"One concern is the effect of repeated delivery of modified RNAs to somatic cells. The cells undergo a rather strenuous treatment regimen of exposure to non-native biological molecules, whose effect is generally not known," said Emile Nuwaysir, VP and COO of **Cellular Dynamics International Inc.**, a producer of human cells derived from iPS cells.

Helen Blau, professor of microbiology and immunology at **Stanford University School of Medicine** and director of the **Baxter Laboratory in Stem Cell Biology**, had similar concerns. "I think that adding genetic material, whether DNA or RNA, still is a risk to iPS cells because like DNA, RNA could also lead to mutations and to immune responses," she said.

Nuwaysir did say he was impressed with the serial mRNA delivery method that Rossi's group described in the paper. "Since the different RNAs can be directly delivered on a precise schedule, it may be possible to discern the stepwise, discrete roles of each of the reprogramming factors," he said.

"The standard method to generate iPS cells for clinical applications has yet to be established. I think this method has the potential for it," concluded Yamanaka, senior investigator at the **Gladstone Institute of**

Cardiovascular Disease and a professor of anatomy at the **University of California, San Francisco**.

Rossi did not disclose patenting or licensing information related to the findings described in the paper.

Baas, T. *SciBX* 3(41); doi:10.1038/scibx.2010.1226
Published online Oct. 21, 2010

REFERENCES

1. Warren, L. *et al. Cell Stem Cell*; published online Sept. 30, 2010; doi:10.1016/j.stem.2010.08.012
Contact: Derrick J. Rossi, Harvard Medical School, Boston, Mass. e-mail: rossi@idi.harvard.edu
2. Takahashi, K. & Yamanaka, S. *Cell* **126**, 663–676 (2006)

COMPANIES AND INSTITUTIONS MENTIONED

Baxter Laboratory in Stem Cell Biology, Stanford, Calif.
Cellular Dynamics International Inc., Madison, Wis.
Gladstone Institute of Cardiovascular Disease, San Francisco, Calif.
Harvard Medical School, Boston, Mass.
Massachusetts General Hospital, Boston, Mass.
Massachusetts Institute of Technology, Cambridge, Mass.
ModeRNA Therapeutics, Cambridge, Mass.
Stanford University School of Medicine, Stanford, Calif.
University of California, San Francisco, Calif.