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Breaking through Barriers; Building Better Cells

Gladstone scientists—led by Nobel Laureate Shinya Yamanaka—have gained new insight into the molecular roadblocks that prevent efficient iPS cell production.

BY ANNE D. HOLDEN, PHD

November 14, 2013—In 2007, Gladstone Institutes' Investigator Shinya Yamanaka, MD, PhD, announced to the world something astounding: the recipe for a chemical 'cocktail' that could transform adult human skin cells into pluripotent cells, similar to embryonic stem cells. He called these cells *induced pluripotent stem cells*, or iPS cells.

Five years later, his efforts had also begun to transform the field of regenerative medicine, and he was awarded the Nobel Prize. But Dr. Yamanaka and his colleagues around the world are still working to understand aspects of the reprogramming process, in the hopes that they can improve the technology. For example, scientists don't yet know the precise series of molecular reactions that transform a skin cell into a stem cell. Also, current methods have a low transformation efficiency, which results in many incompletely reprogrammed iPS cell colonies.

But in the latest issue of the journal *Cell Stem Cell*, Dr. Yamanaka and his Gladstone research team have found a culprit: a family of RNAs that acts as a barrier, preventing adult cells from being completely reprogrammed into iPS cells. And in so doing, the team has also identified a potential way of punching through that barrier and improving stem cell production.

Needle in a Haystack

It takes just four genes, together known as OSKM, to transform a skin cell into an iPS cell. Dr. Yamanaka and his colleagues found that injecting OSKM into an adult skin cell, called a *fibroblast*, induces genome-wide changes within the cell that, over time, reprogram that cell back into a pluripotent state.

"Scientists had always thought that the growth and differentiation of stem cells into various cell types was a one-way street—that once a stem cell became a heart cell, or a brain cell, it couldn't go back," explained Dr. Yamanaka, who also directs the Center for iPS Cell Research and Application at Kyoto University. "But the OSKM 'cocktail' gives us the ability to take the cell on a journey back in time."

However while researchers, armed with OSKM, now have the ability to reprogram cells, the actual process is not so straightforward. For every 100 fibroblasts that are given the OSKM cocktail, at most only one will be successfully reprogrammed. Dr. Yamanaka and his team hypothesized that there were other factors, active during the early stages of reprogramming, which were preventing the cells from transforming completely. So they set out to find them.

"Trying to sift through the factors preventing efficient cellular reprogramming may seem like hunting for a molecular needle in a haystack, so we decided to focus our efforts on a type of molecule called *microRNAs*," said Kathleen Worringer, PhD, the paper's lead author. "Several types of microRNAs are active in fibroblasts, but completely absent in stem cells. So we hypothesized that their activity was serving as a barrier to transformation."

MicroRNAs, or miRNAs, are small, noncoding bits of genetic material that guide how and when certain genes will be switched on and off during the cell's entire lifetime. Of the more than 1,000 distinct miRNAs in the human genome, the research team focused their efforts on a family of miRNAs together known as *let-7*. Previous research in animal models had revealed that *let-7* promoted the differentiation of stem cells, and that cells lacking *let-7* failed to differentiate—they remained stuck in a stem cell-like state. The team reasoned that since *let-7* appears to *promote* differentiation, its presence might also serve as a barrier to transforming a fibroblast into a pluripotent stem cell.

Mapping the Path to Pluripotency

Drs. Worringer and Yamanaka began by adding a new ingredient to the OSKM cocktail: a *let-7* inhibitor. And when they injected this new and improved cocktail into human fibroblasts in a dish, they noticed a distinct uptick in reprogramming efficiency.

The scientists now knew that *let-7* represented a barrier to reprogramming, and that shutting it off allowed the OSKM genes to more efficiently induce reprogramming. But the next question the researchers asked was how. And as so often happens in biology, it was not the removal of *let-7* itself that promoted reprogramming—but rather the chain reaction that occurred as a result.

"Our experiments revealed that inhibiting *let-7* activated a gene called LIN-41 which normally would remain in the 'off' position," said Dr. Worringer, who is also a California Institute for Regenerative Medicine postdoctoral scholar. "This discovery allowed us to pin down the first step in the chain reaction, but what really intrigued us were the steps that followed."

Normally in fibroblasts, *let-7* helps to keep LIN-41 inactive. But with *let-7* removed, LIN-41 comes to life, where it targets a gene called EGR1—and then proceeds to shut it down.

"EGR1 is a 'growth response gene,' which means that it is responsible for regulating the growth and differentiation of cells," continued Dr. Worringer. "Our experiments revealed EGR1 to be a significant obstacle to reprogramming—and that when LIN-41 shut down EGR1, that obstacle was removed."

These findings help clear up the long-standing mystery of what prevents the efficient conversion of fibroblasts into pluripotent stem cells—and offer renewed optimism for scientists and clinicians. They also highlight the complex series of molecular 'pathways' that guide the growth and differentiation of stem cells—and underscore the difficulty of finding ways to reverse that pathway.

And while there is much still to learned, such as the underlying molecular mechanism that causes LIN-41 to target and deactivate EGR1, Dr. Yamanaka is encouraged by these new findings.

"Ever since we first described our ability to generate iPS cells, we've been searching for ways to improve upon it," said Dr. Yamanaka. "Our results represent one very important step towards that goal."