New biotechnology for high efficiency purification of live human cells

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Cell therapies require a purification step that isolates the desired cell types from contaminating cells. Normally cell surface receptors are used as markers to distinguish cell types, but undesired cell types also show these receptors, compromising purification. Evidence suggests microRNA may be a better marker. New biotechnology, miRNA switches, purifies different cell types based on miRNA markers at levels suggesting applicability to patient care.

One of the reasons pluripotent stem cells are so popular in medical research is that they can be differentiated into any cell type. However, typical differentiation protocols lead to a heterogeneous population from which the desired type must be purified. Normally, antibodies that react to surface receptors unique to the desired cell are used for this purpose. However, in many cases the purification levels remain poor and the cells can be damaged. New RNA technology produced at CiRA may avoid these problems.

Professor Hirohide Saito at the Dept. of Reprogramming Science is a bioengineer who makes tools for iPSC researchers. His latest technology, the microRNA (miRNA) switch, is designed to detect and sort live cells not by surface receptors, but by miRNAs. miRNA is a better marker of cell types and can therefore improve purity levels. His miRNA switches consist of synthetic mRNA sequences that include a recognition sequence for miRNA and an open reading frame (ORF) that codes a desired gene, such as a regulatory protein that emits fluorescence or promotes cell death. If the miRNA recognition sequence binds to

miRNA expressed in the desired cells, the expression of the regulatory protein is suppressed, thus distinguishing the cell type from others that do not contain the miRNA and express the protein.

Senior Lecturer Yoshinori Yoshida is a cardiomyocyte specialist also at the Dept. of Reprogramming Science who immediately saw the potential of this technology. He has been studying how iPS cells can be used to combat cardiac diseases, but has been stymied by unsatisfactory purity levels. Cardiomyocytes are especially difficult to purify because they do not express unique surface receptors. The two scientists therefore collaborated to investigate the effectiveness of miRNA switches for these cells.

By applying the miRNA switches to a defined cardiomyocyte cell line, they were able to identify miRNAs unique to cardiomyocytes. From there, miRNA switches that contained sequences complementary to these miRNAs were constructed. The result was far better purification than that achieved by standard methods. Furthermore, because this technology is RNA-based, it does not integrate into the genome, which makes the cells eligible for clinical application.

Yoshida sees this tool as remarkably simple and something that can be used by stem cell researchers studying any organ. "It is just synthesizing RNA and transfecting them. It is not difficult," he said. To prove this point, he and Saito applied their miRNA switches to the purification of hepatocytes and pancreatic cells, neither of which has unique cell surface markers, finding miRNA switches effective there too.

Intriguingly, the performance of different miRNA switches varied with cell development, suggesting that strategic selection of miRNAs could separate cardiomyocytes at different developmental stages and lead to an even more homogeneous cell pool and potentially better cell therapy outcomes.

Saito believes that with further development, miRNA switches will be applicable to all cell types at all cell stages. "We want to make an active miRNA dictionary for each cell type, so that if we want to isolate this kind of cell type, we know how to use this kind of switch," he said.

The study is published in the *Cell Stem Cell*.

More information: "Efficient detection and purification of cell populations using synthetic microRNA switches." *Cell Stem Cell*, 2015.

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