Human embryonic stem cells purified in new, rapid technique

August 6 2010

(PhysOrg.com) -- UCSF researchers are reporting the first success in very rapidly purifying one type of embryonic stem cell from a mix of many different types of embryonic stem cells in the culture dish. The technique, which avoids the need to genetically alter the cells to distinguish them, is a key advance, the researchers say, for obtaining the appropriate cells for repairing specific damaged tissues.

The new strategy links two existing technologies for the first time: the ability to identify specific embryonic stem cell types in a culture of different embryonic stem cells, and a way to efficiently sort them at a very high rate, a procedure known as “high throughput” processing.

“Before stem cell therapy can become routine, clinicians will need a plentiful and certain supply of pure stem cells that is capable of forming the particular tissue to be repaired, and is free of contamination by other cell types. But the goal of rapidly and safely harvesting vast numbers of a single stem cell type without altering the cell’s genome has been challenging,” says Harold Bernstein, MD PhD, UCSF professor of pediatrics, a member of the UCSF Cardiovascular Research Institute and senior author of the paper.

“Here we were able to purify one specific cell type without resorting to genetically engineering the stem cells themselves, a process that can introduce unwanted traits into the cells.”

The research finding is currently published online in the journal *Stem*

Cells and Development, and will appear later this year in a print edition of the journal. Embryonic stem cells, which replicate indefinitely in the culture dish, are capable of forming almost any tissue in the body. Over time, they begin to specialize as specific cell types, such as cardiomyocytes of the heart or neurons of the brain. One goal for stem cell therapy is to be able to identify cells that have begun to specialize in a particular way so that they could serve as a source of cells to repair specific damaged tissues.

While embryonic stem cell cultures are made up primarily of cells that have begun to differentiate, they also include cells that remain unspecialized, and thus have the capacity to form tumors, called teratomas. Scientists have attempted to purify stem cells—whether to eliminate those with the potential to form teratomas or to isolate specific embryonic stem cell types—by using viruses to insert DNA into the stem cells' genes. This technique allows researchers to distinguish one type of cell from another, but this genetic engineering approach carries the risk of altering the natural makeup of the stem cells.

The UCSF scientists used a different strategy. They identified cells that can form teratomas by searching for a telltale snippet of DNA in the tumor cells' genes. They chemically tagged these cells without altering them, and the cells were then removed on the basis of this temporary molecular tag. They reported separating out the desired stem cells from the teratoma-forming cells at a rate of about 25,000 cells per second.

The researchers say they expect the same approach could be used to separate and purify different types of cells as they advance from the stem cell state into neurons, heart cells or any other type of tissue needed for future stem cell therapy.

"Stem cell therapy requires us to select the cells we need, to eliminate teratoma-forming cells from the desired stem cells, and to accomplish

this in a high-throughput manner so that we can obtain enough cells," says Bernstein. "We show how all three goals can be accomplished at once."

"We envision this as a tool that ultimately could rapidly identify and purify many different kinds of differentiating cells on their way to becoming heart muscle or pancreas or skin cells. This approach could quickly build up a large reservoir of desired cells," he said.

The research was supported in part by the state-funded California Institute for Regenerative Medicine, or CIRM. The technique has been disclosed to the UCSF Office of Technology Management for potential licensing, as well as to CIRM.

In the technique reported, the scientists introduced into a stem cell culture a pair of DNA snippets containing part of a gene, called Oct4, which is essential to all embryonic stem cells. The gene also allows cells to form teratomas, but it is not active once a stem cell starts down the road to becoming a specialized cell type.

At one end of each snippet, the researchers attached a fluorescent protein, and, at the other end, they engineered the DNA so that it could "quench" the fluorescent light. When introduced into the cell culture, the two DNA fragments naturally sought to bind, or anneal, to similar genetic sequences in the messenger RNA, or mRNA, made by the cell's Oct4 gene. However, they could do so only if the gene was turned on, as it is in teratoma-forming cells—not in stem cells already destined to become specialized tissue.

When the two DNA snippets annealed to an Oct4 mRNA, their fluorescent proteins lit up, and a cell-sorting machine then fished out these the fluorescently tagged cells—but no others.

“The key to the process,” says Bernstein, “is that the two fluorescently tipped fragments light up only when they are close to each other, and they are only close to each other when they both anneal to a target cell’s Oct4 genetic sequence.” If Oct4 is not active in a cell, then the two introduced DNA strands do not light up. Cells that lack a fluorescent tag can then be automatically sorted separately from the fluorescently tagged target cells.

This approach enabled the researchers to isolate the teratoma-forming cells from stem cells that were already on their way to becoming more specialized cells and thus no longer expressed the telltale Oct4 gene product. The scientists expect that other tags can be developed to separate different kinds of developing cells.

The targeted gene in the process is one that codes for the Oct4 transcription factor, which allows embryonic stem cells to remain pluripotent. The DNA snippets are called “molecular beacons,” and the tagging part of the two-step strategy in which the two snippets interact is known as fluorescence-resonance energy transfer, or FRET. Molecular beacons were first described in 1998 for use in DNA sequence analysis, and cell sorters have been used for decades, but linking them represents a new technology for identifying and purifying special types of stem cells. The use of the cell sorter also allows for high throughput, or the processing and purifying a large number of special cells in a short period of time.

Lead author on the paper is Frank W. King, PhD, who was a postdoctoral fellow with Bernstein at the UCSF Cardiovascular Research Institute when this research was done. Co-authors on the paper are Walter Liszewski, BA, and Carissa Ritner, BS, both staff research associates in the Bernstein Lab at the CVRI.