New discovery may eliminate potentially lethal side effect of stem cell therapy

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Like fine chefs, scientists are seemingly approaching a day when they will be able to make nearly any type of tissue from human embryonic stem cells. You need nerves or pancreas, bone or skin? With the right combination of growth factors, skill and patience, a laboratory tissue culture dish promises to yield therapeutic wonders. But within these batches of newly generated cells lurks a big potential problem: Any remaining embryonic stem cells - those that haven't differentiated into the desired tissue - can go on to become dangerous tumors called teratomas when transplanted into patients.

Now researchers at the Stanford University School of Medicine have developed a way to remove these pluripotent human embryonic stem cells from their progeny before the differentiated cells are used in humans. ("Pluripotent" describes cells that are able to become all types of adult tissue.)

"The ability to do regenerative medicine requires the complete removal of tumor-forming cells from any culture that began with pluripotent cells," said Irving Weissman, MD, director of the Stanford Institute for Stem Cell Biology and Regenerative Medicine. "We've used a combination of antibodies to weed out the few undifferentiated cells that could be left in the 10 or 100 million differentiated cells that make up a therapeutic dose."

Weissman pointed out that the production of therapeutic cells from pluripotent stem cells for regenerative medicine was a major goal of

Proposition 71, the ballot measure that established the California Institute for Regenerative Medicine to allocate $3 billion to advance stem cell science. CIRM funded this research.

The scientists believe the technique could also be used to remove residual tumor-initiating cells from populations of cells derived from induced pluripotent stem, or iPS, cells. These cells may also be useful for therapy but, unlike embryonic stem cells, iPS cells are created in the laboratory from adult tissue.

"Commonly used differentiation protocols for embryonic stem and iPS cells often give rise to mixed cultures of cells," said research associate Micha Drukker, PhD. "Because even a single undifferentiated cell harbors the ability to become a teratoma, we sought to develop a way to remove these cells before transplantation."

Drukker is the senior author of the research, which will be published online Aug. 14 in *Nature Biotechnology*. Stanford medical student Chad Tang is the first author. Weissman, who is also the Virginia and D.K. Ludwig Professor for Clinical Investigation and Cancer Research and a member of the Stanford Cancer Institute, is a co-author. The research was conducted in his lab.

Teratomas are the Frankensteins of the tumor world - a hodgepodge of tissues like teeth, hair and bone. They owe their remarkable composition to the fact that the cells from which they arise early in development are pluripotent. In fact, the ability to form teratomas in animals is a defining feature of true pluripotent cells.

But the very feature that confirms a cell's pluripotency also makes it potentially dangerous to use therapeutically. That's why Tang, Drukker and Weissman decided to try to develop an antibody that would recognize and bind to only pluripotent cells and enable their removal

from a mixture of cells. Although a few such antibodies already existed, they were not specific enough on their own to completely weed out the tumor-causing cells.

The researchers studied two sets of antibodies - one commercially available and one they generated themselves - to identify which among them bound most strongly to pluripotent, but not differentiated, cells. They found one newly generated antibody that was highly specific for a previously unknown marker on undifferentiated cells that they termed stage-specific embryonic antigen-5, or SSEA-5. The cells bound by this antibody, anti-SSEA-5, expressed high levels of pluripotent-specific genes and resembled embryonic stem cells in appearance. Anti-SSEA-5 also bound strongly to the inner cell mass of an early human embryo, the group of cells from which embryonic stem cell lines are derived.

When the researchers injected human embryonic stem cells recognized by anti-SSEA-5 into mice, they found that in seven out of seven times, the cells formed rapidly growing teratomas. However, cells that were not bound by anti-SSEA-5 formed smaller teratomas in only three of 11 experiments. Combining anti-SSEA-5 with two other antibodies known to bind to pluripotent cells completely separated the pluripotent from the differentiated cells, although the researchers did see some smaller, less-diverse growths in some cases.

Upon analysis, Tang and his colleagues found that anti-SSEA-5 recognizes and binds to a cell-surface carbohydrate structure called a glycan. As the pluripotent cell differentiates, this glycan is modified to other glycan structures not recognized by the antibody.

"The study of glycans is becoming an active area of stem cell biology," said Tang. "Many glycans are highly expressed in embryonic stem cells, but not in differentiated cells. This warrants further study and may lead to new understandings about embryonic stem cell biology."

In addition to Tang, Drukker and Weissman, other Stanford researchers who participated in the study include graduate student Andrew Lee; postdoctoral scholar Jens-Peter Volkmer, MD; instructor of pathology Debashis Sahoo, PhD; undergraduate student Divya Nag; research assistant Adriane Mosley; research associate Matthew Inlay; instructor of medicine Reza Ardehali, MD; postdoctoral scholar Shawn Chavez, PhD; professor of obstetrics and gynecology and director of Stanford's Human Embryonic Stem Cell Research and Education Center Renee Reijo Pera, PhD; professor of obstetrics and gynecology Barry Behr, PhD; and associate professor of cardiovascular medicine and of radiology Joseph Wu, MD, PhD.

Stanford University has filed for patent protection for the use of monoclonal antibody-based protocols to remove teratogenic pluripotent stem cells from a cell mixture.

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