Priming Embryonic Stem Cells To Fulfill Their Promise

January 21, 2005

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Bioengineering researchers at the University of California, San Diego have invented a process to help turn embryonic stem cells into the types of specialized cells being sought as possible treatments for dozens of human diseases and health conditions. Sangeeta Bhatia and Shu Chien, UCSD bioengineering professors, and Christopher J. Flaim, a bioengineering graduate student, described the cell-culture technique in a paper published in the February issue of *Nature Methods*, which became available online on Jan. 21.

Embryonic stem cells are considered the blank-slate, raw material needed to repair or replace damaged or missing liver, nerve, muscle, and other tissues and organs. However, in order to fulfill their therapeutic promise, scientists believe that stem cells must first be coaxed to differentiate, or mature, into precursors of specialized cells found in the body.

Embryonic stem cell differentiation is complex and far from fully understood. Scientists are focusing on four types of external inputs known to be involved in triggering the differentiation of stem cells: soluble growth factors, adjacent cells, mechanical forces, and extracellular matrix proteins that form the support structure of almost all tissues. Bhatia, Chien, and Flaim focused on just one - the extracellular matrix.

"We kept the other factors constant and developed a miniaturized technique to precisely vary extracellular matrix proteins as a way to identify which combinations were optimal in producing differentiated cells from stem cells," said Bhatia. She, Chien, and Flaim described in their paper a technique that enabled them to identify the precise mix of proteins that optimally prompted mouse embryonic stem cells to begin the differentiation process into liver cells. Bhatia, Chien, and Flaim designed the technique with other cell biologists in mind so that any of them could duplicate it with off-the-shelf chemicals and standardized laboratory machinery. "We think other researchers could easily use this technique with any other tissue in mouse, or human, or any other species," said Bhatia.

Scientists have identified about 100 proteins - including laminin, fibronectin, and several kinds of collagen - that function as the extracellular matrix, or scaffolding, of most mammalian tissues. Until now, there has been no practical way to evaluate the many possible combinations of these proteins, any one of which could form the optimal "niche" for a desired type of differentiated cell.

In their experiments, the UCSD researchers took advantage of the knowledge that the extracellular matrix in liver is comprised primarily of just five proteins. They applied spots of all 32 possible combinations of the five proteins as engineered niches onto the surface of gel-coated slides, and then added mouse embryonic stem cells to the niches. After the cells were allowed to grow, the researchers assayed their progression into liver cells. "We looked at all the combinations at once," said Bhatia. "Nobody has done this combinatorial approach before."

Bhatia, Chien, and Flaim reported that either collagen-1 or fibronectin had strongly positive effects on the differentiation of the stem cells they tested. Unexpectedly however, when both collagen-1 and fibronectin were combined in one niche, the liver cell differentiation process was subtly inhibited. "You would not predict that from the customary cell biology experiments," said Bhatia. "By using this combinatorial technique we were surprised

to find many interesting interactions, and we were able to tease out the effects of each protein, alone and in combination with others."

Cell biologists have not performed such combinatorial assays for other desired cell types because they had no practical way to do so. Bhatia, Chien, and Flaim seized on the unique ability of so-called DNA spotting machines to deliver tiny volumes of liquid, about one trillionth of a liter per spot. The spotting machines, which cost about $20,000, have become common fixtures at most research universities, but the innovation reported today in *Nature Methods* involved using such a machine to spot solutions of proteins rather than DNA. The UCSD researchers also refined other parameters so that the technique would be reproducible in other research laboratories.

"When we talked to our colleagues, it was clear that, whether it's cells in the liver, brain, or heart, there had been no practical way for researchers to find the optimal extracellular matrix needed to turn embryonic stem cells into cells with therapeutic potential," said Bhatia. "We think we've developed an enabling technology for stem cell research and other areas of cell biology in the sense that all of a sudden scientists can use inexpensive and widely available reagents and machinery to optimize the conditions needed to optimize embryonic stem cell differentiation."

Bhatia is planning further studies on generating liver cells from embryonic stem cells to make an artificial liver. She plans to seek funding to further her artificial liver research from the new California Institute for Regenerative Medicine. The institute was created after California voters in November approved Proposition 71, a measure that authorized the state to borrow $3 billion to fund stem cell research over the next 10 years.

"I'm really excited about the stem cell applications of our new technology," said Bhatia. "We feel that this extracellular matrix part of the stem cell niche has been understudied. If we can now take what we've learned, add combinations of growth factors, and even add other cells to embryonic stem cells, we may be able for the first time to study at all the dimensions of the niches required to very specifically control embryonic stem cell differentiation."

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