# Scientists solve mystery of fragile stem cells

April 12 2010

Scientists at The Scripps Research Institute have solved the decade-old mystery of why human embryonic stem cells are so difficult to culture in the laboratory, providing scientists with useful new techniques and moving the field closer to the day when stem cells can be used for therapeutic purposes.

The research is being published in the journal Proceedings of the National Academy of Sciences during the week of April 12, 2010.

"This paper addresses a long-standing mystery," said Scripps Research Associate Professor Sheng Ding, who is senior author of the paper. "Scientists have been puzzled by why human embryonic stem cells die at a critical step in the culture process. In addition to posing a question in fundamental biology, this created a huge technical challenge in the lab."

The new paper, however, provides elegant solutions to both aspects of this problem.

In the study, the team discovered two novel synthetic small molecule drugs that can be added to human stem cell culture that each individually prevent the death of these cells. The team also unravels the mechanisms by which the compounds promote stem cell survival, shedding light on a previously unknown aspect of stem cell biology.

## Notorious Fragility

The hope of most researchers in the field is that one day it will be

possible to use stem cells — which possess the ability to develop into many other distinct cell types, such as nerve, heart, or lung cells — to repair damaged tissue from any number of diseases, from Type 1 diabetes to Parkinson's disease, as well as from injuries.

Laboratory work with human embryonic stem cells, however, has been hampered by their notorious fragility. In the process of growing stem cells in culture, scientists must split off cells from their cell colonies. At this point in the process, however, human embryonic stem cells die unless the scientists take extraordinary care that this does not happen.

"The current techniques to keep these cells alive are tedious and labor-intensive," said Ding. "Keeping the cells alive is so difficult that some people are discouraged from entering the field. It is very frustrating experience for everyone."

Mysteriously, mouse embryonic stem cells—which share much basic biology with human embryonic stem cells—do not pose the same difficulties in the laboratory. They can usually be split off from a colony and go on to survive and thrive.

To address these issues, the scientists decided to start with a screen of a library of chemical compounds to see if they could find any small molecules that could be added to the human embryonic stem cell culture that would promote the cells' survival.

When the scientists examined their results, they were elated to find two novel compounds (named Thiazovivin and Pyrintegrin) that both worked to dramatically protect the cells, promoting human embryonic stem cell survival by more than 30 fold.

"Basically, this solved this cell survival problem that has been plaguing scientists for more than 10 years," said Ding.

## The Importance of Interaction

But the scientists didn't stop there.

Next, using the two new survival-promoting small molecules as clues, the scientists set out to understand the biological mechanism behind the cells' survival or demise. By examining cell growth in the presence and absence of the compounds, the team found that the key factor was a protein on the cell surface called e-cadherin, which mediates interactions among cells and between cells and the extracellular matrix (a structure present between a variety of animal cells that provides support and anchorage for cells and regulates intercellular communication).

"While in the past people have often talked about the proteins in cell nucleus as regulating stem cell function, our study puts the focus on a different area," said Ding. "E-cadherin is a protein on the cell surface that is very important to cell survival and cell growth."

The team found that when human embryonic stem cells are cut out from the colony, this key protein is disrupted and then internalized within the cell. Without e-cadherin on the cell surface, cell signaling between the cells and their environment is disrupted and the cells quickly die.

Both chemical compounds identified by the study, however, protected e-cadherin from damage.

In further experiments, the scientists found that the key difference between human and mouse embryonic stem cells lay not only within the cells themselves, but also in and controlled by their microenvironment—the surrounding cells, signaling factors, and extracellular matrix. The scientists were able to transfer human embryonic stem cells into a mouse embryonic stem cell microenvironment. There, the scientists found, human cells were more

likely to survive, even without the survival-promoting compounds.

Moreover, when the scientists chemically induced human embryonic stem cells back to an earlier stage of development—which had an extracellular environment similar to mouse embryonic stem cells conventionally used in the laboratory—there were also no longer problems growing them in culture.

"This validated our mechanistic investigations from a different angle," said Ding, "showing that we had dissected out a very core regulatory mechanism."

Ding expects that the methods discussed in the new study will soon be widely adopted by stem cell laboratories around the world.

"My lab currently uses the novel small molecules indentified in this study on a routine basis, making our life significantly easier and advancing our efforts," said Ding. "Even more, chemically inducing human embryonic stem cells back to an earlier stage of development has advantages for some areas of investigation."

**More information:** Yue Xu et al., "Revealing a core signaling regulatory mechanism for pluripotent stem cell survival and self-renewal by small molecules," *Proceedings of the National Academy of Sciences*.

Provided by The Scripps Research Institute