Researchers find solution to cell death problem vexing stem cell research

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Human pluripotent stem (hPS) cells can generate any given cell type in the adult human body, which is why they are of interest to stem cell scientists working on finding therapies for spinal cord injuries, Parkinson's disease, burns, heart disease, diabetes, arthritis, and other ailments.

Before hPS cell technologies can be translated into clinical applications, however, some obstacles must first be overcome.

One such obstacle frustrating stem cell researchers is "cell death" that the major types of hPS cells, including human embryonic stem cells and human induced pluripotent stem cells, mysteriously undergo when cultured as single cells, rendering them less suitable for research.

Researchers at the University of California, Riverside now show that a molecular motor, called "nonmuscle myosin II" (NMII), which exists naturally inside each hPS cell and controls various cellular functions, triggers the death of hPS cells when they are broken down to single cells.

While many details of how exactly NMII works remain unknown, a wide consensus among researchers is that NMII induces a contraction of the main internal components of the cells, eventually resulting in cell death.

To stop this cell death, the researchers treated hPS cells with a chemically synthesized compound, blebbistatin, and found that it substantially enhanced the survival of the cells by chemically inhibiting

NMII. (Blebbistatin is commercially available from several companies that sell biologically active chemical compounds.)

"Our research shows that blebbistatin works as effectively as the most potent cell death inhibitor of hPS cells available today," said Noboru Sato, an assistant professor of biochemistry, whose lab led the research. "This discovery brings stem cell research a step closer towards finding therapies for several diseases."

Study results appear online, Sept. 7, in *Nature Communications*.

Sato explained that most of the current culture methods to grow hPS cells require animal-derived materials, such as Matrigel, for coating the culture surfaces. Without these materials, hPS cells cannot adhere to the culture plate. But the drawback of using them is that they could potentially cause contamination of hPS cells by introducing viruses and unknown pathogens.

"Another advantage of using blebbistatin is that we need no human- or animal-derived materials for coating the culture surfaces," he said. "This is because blebbistatin greatly facilitates the adhesion of cells to the culture surface. By combining blebbistatin and a chemically synthesized coating material, poly-D-lysine, we have developed a fully defined and simplified culture environment that allows hPS cells to grow under completely animal-free and contamination-free conditions."

Available through many companies, poly-D-lysine is a chemically synthesized animal-free coating material that is widely used for cell culture coating for other cell types. For hPS cells to adhere to the poly-D-lysine coating, blebbistatin must be added to the culture medium. "This new method shows that a novel combination of routinely available materials can create a completely distinct technological platform," Sato said.

Provided by University of California - Riverside