Identifying stem cell suicide triggers with ROCK

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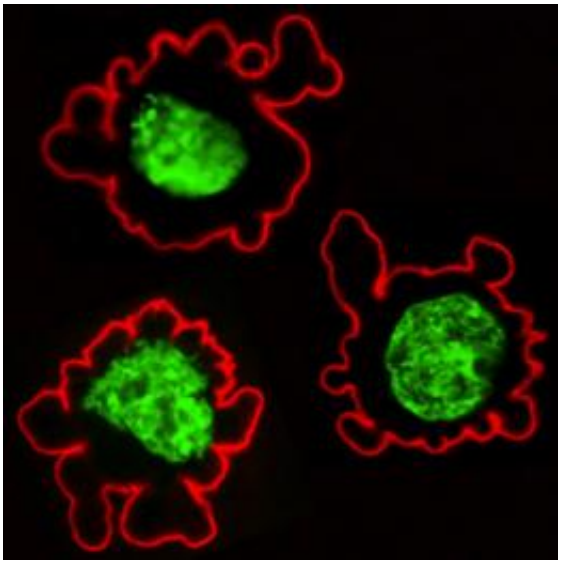

Fluorescent imaging of blebbing in live, dissociated human stem cells. The plasma membrane is red, and the nucleus is green. Copyright : 2010 Yoshiki Sasai

A RIKEN-led team of molecular biologists (Japan) has determined why human embryonic stem cells (ESCs) and induced pluripotent stem (iPS) cells undergo apoptosis, or programmed cell death, when separated from each other. The finding should allow more efficient culturing of human stem cells, making them easier to maintain, more flexible to handle, and generally improving their survival.

At present, human ESCs, unlike those derived from mice, must be cultured in clumps, which makes them difficult to manipulate. When they lose contact with neighboring cells, human ESCs immediately go into apoptosis.

The research team, led by Yoshiki Sasai from the RIKEN Center for Developmental Biology in Kobe, and including members from Kyoto University, showed that this apoptotic response could be countered by application of an inhibitor of the enzyme ROCK (Rho-dependent protein kinase). Now, using a combination of live-cell imaging and laboratory analysis, the team has elucidated the onset and progress of dissociation-induced apoptosis1.

They found that within a few hours of separation, human ESCs began blebbing—a process whereby the membrane spontaneously bulges in finger-like projections causing the cells to jiggle around. Blebbing occurs when the membrane breaks away from the internal cytoskeleton, and can vary in its duration and severity. In this case, blebbing lasted for hours and inevitably ended with the cell bursting. The researchers dubbed it the death dance, and traced its onset to hyperactivation of myosin, a contractile protein associated with cell movement.

By studying the levels of ROCK after dissociation, as well as the regulation of its activity by the compounds with which it interacts, Sasai and colleagues determined that myosin hyperactivation—hence the blebbing and apoptosis—is caused directly by ROCK. It can be suppressed by the myosin inhibitor, blebbistatin. Further, the whole process is triggered by loss of intercellular contact, and regulated by a compound known as Abr.

The molecular mechanism that the researchers have unraveled should be susceptible to manipulation, potentially allowing human ESCs to be separated and handled without risking their certain death. Interestingly,

they found that the difference in susceptibility to apoptosis of dissociated human and mouse ESCs had nothing to do with species, but could be attributed to the stage of development from which the parent stem cells were derived.

“We are now planning further work to understand the detailed mechanism of Abr activation,” says Sasai. “Another question we wish to study is why cells die upon myosin hyperactivation.”

More information: Ohgushi, M., et al. Molecular pathway and cell state responsible for dissociation-induced apoptosis in human pluripotent stem cells. *Cell Stem Cell 7*, 225–239 (2010). www.cell.com/cell-stem-cell/abstract/S1934-5909%2810%2900333-4

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