STEM CELLS

Throwing a ROCK inhibitor at a problem

In a screen to improve human embryonic stem cell (hESC) survival during culture, researchers found that an inhibitor of a newly identified kinase did the job.

One of the biggest obstacles in hESC research is the poor survival rate of these cells after dissociation. As cell dissociation is key for subcloning as well as for induction of differentiation, researchers at the Center for Developmental Biology at RIKEN sought a way to avoid cell death in hESC culture.

Yoshiki Sasai's group tested the effects of two dozen various enzyme inhibitors, growth factors and other compounds for effects on cell death, growth, differentiation and cell attachment. In their initial screening, "none of the conventional apoptosis inhibitors worked, and therefore we were convinced that this cell death involves some unknown factors," explains Sasai.

With this in mind, they tested Y-27632, a selective inhibitor of a recently identified

Figure 1 | Culture of dissociated hESCs in the absence (left) and in the presence (right) of Y-27632. Scale bars, 500 μ m. Reprinted from *Nature Biotechnology*.

kinase, p160-Rho-associated coiled-coil kinase (ROCK). Upon treatment with this compound, dissociation-induced apoptosis of hESCs was much lower than in untreated cells, and the treated cells produced many large colonies (**Fig. 1**). The cloning efficiencies of untreated and inhibitor-treated cells were 1% and 27%, respectively.

"I was just lucky in finding this effective inhibitor in a small candidate screening. To be honest, I wonder why high-throughput

screens done in many companies or institutes failed to identify this molecule," questions Sasai, postulating that ROCK may have been overlooked because it is a newly identified kinase.

The mechanism of apoptosis inhibition by Y-27632 has yet to be elucidated and may provide clues to why hESCs have a poor survival rate after dissociation. But for now this inhibitor, which is water soluble and stable, can be used while maintaining hESCs as well as during differentiation.

"The best wish of mine is that this method speeds up the translation of the culture technique established with mouse embryonic stem cells into hESC applications," concludes Sasai.

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RESEARCH PAPERS

Watanabe, K. *et al.* A ROCK inhibitor permits survival of dissociated human embryonic stem cells. *Nat. Biotechnol.* **25**, 681–686 (2007).