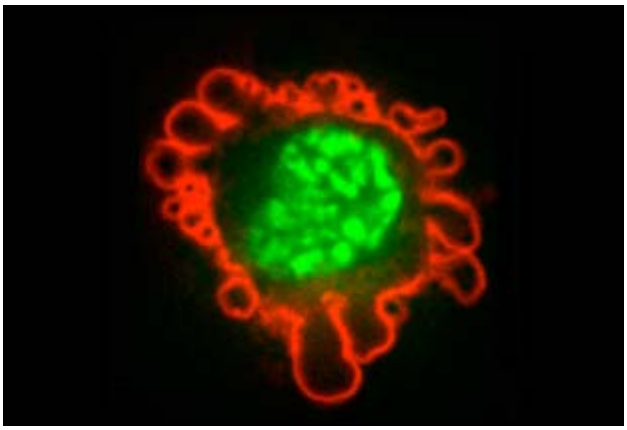
RIKEN Center for Developmental Biology (CDB)
2-2-3 Minatojima minamimachi, Chuo-ku, Kobe 650-0047, Japan

ROCK's role in hESC suicide prevention

August 6, 2010 – Embryonic stem cells (ESCs) are known for their ability to be grown indefinitely in self-renewing culture, and for their pluripotency–the differentiative potential to give rise to all the cell types in the body. These characteristics are common to such cells from both mouse and human, but ESCs from these species also exhibit important differences, one of the most prominent of which is the greater susceptibility to cell death of human ESCs on dissociation; mouse ESCs are made of sturdier stuff, and grow happily even when isolated from their cell-mates. Although methods have been developed to prevent hESCs from self-destructing, including a breakthrough report from the Laboratory for Organogenesis and Neurogenesis (Yoshiki Sasai, Group Director) in 2007, the mechanisms behind its occurrence have never been explained.



Extensive blebbing in dissociated hESC. Nucleus, green; cell membrane, red.

A new study by led by Masatoshi Ohgushi in the same group, in collaboration with the CDB Laboratory for Pluripotent Stem Cell Studies and scientists at Kyoto University, now provides an answer to that puzzle. Published in *Cell Stem Cell*, the authors report that hESC cells die due to a hyperactivation of myosin that is triggered by loss of cell-cell adhesion and mediated by the Rho signaling pathway. These changes in molecular activity are accompanied, but not dependent on, changes in cells morphology known as "blebbing," in which the cells perform a "dance of death" as an early signal of their demise.

Ohgushi began by building on previous work from the same lab, which had shown that inhibition of a component of the Rho signaling pathway known as ROCK can prevent human ESCs from dying on separation from their colony. He used a combination of cell sorting and live imaging to identify features of dying hESCs, and found that quickly after dissociation the cells became motile and began to form distinct outpockets in their surface membranes, called blebs. This symptom is known to be the result of the actomyosin system, which regulates cytoskeletal behavior, but it is ordinarily a short-lived phenomenon, a kind of convulsive last gasp in a dying cell's last minutes; in hESCs, however, it lasts for as long as a day. And interestingly, while blebbing is usually under the control of the caspase pathway that regulates canonical forms of programmed cell death, its onset in hESCs is caspase-independent, but can be prevented by ROCK inhibition.

It turns out that the reason for ROCK's role is its effect on non-muscle myosin light chain 2 (MLC2). ROCK is upregulated following dissociation of hESCs, which triggers an elevation of MLC2, hyperactivating myosin and generating the intracellular contractive forces that underlie blebbing. Interestingly, however, they found that inhibition of blebbing did not prevent apoptosis.

Contact: Douglas Sipp sipp@cdb.riken.jp TEL: +81-78-306-3043
RIKEN CDB, Office for Science Communications and International Affairs

The group's first supposed the true cause of this programmed cell death in hESCs on dissociation would be a phenomenon known as anoikis (from the Greek word for "homeless"), in which cells detached from their basement membrane spontaneously undergo apoptosis. But live imaging showed that even when dissociated hESCs remained attached to the basement membrane, they blebbed and died. It appeared that the loss of lateral, not basal, connections was to blame, suggesting a role for cadherin-mediated cell-cell adhesion. They tested this notion by depleting calcium ions (cadherin function is calcium-dependent) and cadherin knockdown by RNAi in hESCs, and in both cases observed blebbing and apoptosis, suggesting that the loss of intercellular adhesion plays a triggering role in the apoptotic cascade. This jibes well with known differences between the cellular states of mouse and human ESCs; specifically, the similarity of hESCs to mouse epiblast cells.

Loss of cadherin function also induced the upregulation of the Rho signaling pathway, of which ROCK is a component. Previous work has shown that this pathway is subject to multiple upstream activators, so Ohgushi et al. performed a an shRNA knockdown screen to identify responsible factors, and turned up a heretofore poorly characterized Rho-GEF family member known as Abr.

The involvement of Abr cements the importance of Rho signaling in the demise of dissociated hESCs. Importantly, Rho is frequently found to interact with another small G protein, Rac. Using a pulldown assay, Ohgushi looked at the control of this factor in dissociated hESCs, and found that, in contrast to Rho, Rac was significantly downregulated, strongly suggesting that Rho and Rac have reciprocal roles in this process. The take-home message appears to be that loss of cadherin-mediated cell-cell adhesion in dissociated hESCs upregulates Abr, leading to a Rho-high/Rac-low state, consequent elevation of ROCK, and the hyperactivation of myosin, causing blebbing and apoptotic death.

The developmental and evolutionary significance of this mechanism remains a stimulating open question. "In mouse, the cells of the ICM show no polarity, while those of the epiblast do, enabling them to adhere and form a sheet-like structure, and we have seen that dissociated mouse epiblast cells undergo the same apoptosis as do dissociated hESCs," says Sasai. "This dance of death induced by the hyperactivation of myosin is an intriguing cellular phenomenon in the development of a multi-celled 'society' during early embryogenesis."

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