STEM CELLS

## Bend me, shape me…

Two cell types, adipocytes and osteoblasts; one common ancestor, human mesenchymal stem cells (hMSCs). The distinguishing factors? Cell shape, cytoskeletal tension and RhoA–ROCK signalling, according to Christopher Chen's group, who report their findings in *Developmental Cell*.

Confirming previous reports, adipocyte differentiation was favoured when hMSCs were plated at a high density and in adipogenic culture medium, whereas a low plating density and osteogenic factors favoured osteoblastic commitment. Many cellular characteristics are affected by changes in cell densities — for example, increasing cell density decreases spreading and cell–substrate adhesion. So Chen and colleagues explored the effect of cell shape on stem-cell commitment. They generated 'islands' of fibronectin to control the spreading of hMSCs. hMSCs plated on small islands were more rounded, as they couldn't spread, and gave rise to adipocytes; those seeded onto large islands could spread, and generated osteoblasts.

Because cellular shape and cytoskeletal dynamics are intimately linked, the authors examined whether the actin cytoskeleton was somehow involved in the shape-induced commitment process. They disrupted the actin cytoskeleton using an inhibitor of ROCK, Y27632, to inhibit myosin-generated cytoskeletal tension. hMSCs that were seeded at low density, and therefore expected to become osteoblasts, instead expressed adipocyte-specific markers when treated with Y27632, which implies that the actomyosin cytoskeleton influences hMSC commitment.

So, could there be a connection between cell spreading, cytoskeletal tension and cell fate? RhoA regulates cytoskeletal tension in other cell types and so might transduce signals that are induced by cell-shape changes in hMSCs to changes that affect cell fate. To test this, the authors plated cells out at low or high densities (favouring osteogenesis and adipogenesis, respectively) in osteogenic or adipogenic media and measured levels of active RhoA. RhoA activity was highest in low-density cultures in osteogenic media and correlated with increased cell spreading and ROCK activity.

The authors therefore investigated whether RhoA could directly influence cell-fate choices in hMSCs. In culture medium lacking differentiating factors, hMSCs infected with constitutively active RhoA became osteoblasts, whereas those infected with dominant-negative RhoA formed adipocytes. Conversely, dominant-negative RhoA blocked osteogenesis that was induced by placing the cells in osteogenic media, and encouraged these cells to adopt an adipogenic fate; and constitutively active RhoA inhibited adipogenesis in cells that were given adipogenic media and redirected them to become osteoblasts. So RhoA can replace soluble-factor signalling, but this depends on its ability to affect the cytoskeleton; disrupting the actin cytoskeleton by inhibiting ROCK or myosin abrogated the ability of constitutively active RhoA to induce osteogenesis.

But manipulating RhoA signalling did not similarly redirect hMSC commitment in response to cell shape. Cells plated on small islands, which were therefore round and expected to become adipocytes, could not be persuaded to become osteoblasts when infected with constitutively active RhoA, whereas adipogenesis was blocked simply by inducing cell spreading, even when dominant-negative RhoA was expressed. So in this case, cell shape and RhoA activity are required to specify hMSC cell fate. By contrast, constitutively activated ROCK seemed to control the cells' fate regardless of their shape, implying that ROCK functions downstream of both soluble factors and cell shape in regulating hMSC commitment.

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**References and links**

**ORIGINAL RESEARCH PAPER** McBeath, R. *et al.* Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev. Cell* **6**, 483–495 (2004)

# IN BRIEF

CELL DEATH

### Alkylating DNA damage stimulates a regulated form of necrotic cell death.

Zong, W.-X. *et al. Genes Dev*. **18**, 1272–1282 (2004)

Necrosis has been regarded as an unregulated form of cell death. But, Craig Thompson and colleagues now show that this is not the case. They found that alkylating agents induce necrotic cell death in apoptosis-deficient cells, and that this requires the activation of the DNA-repair protein poly(ADP-ribose) polymerase (PARP). In cells that use mainly aerobic glycolysis for their ATP production — such as proliferating cells, including cancer cells — PARP triggers the depletion of ATP, which causes subsequent necrosis.

CELL CYCLE

### A role for the FEAR pathway in nuclear positioning during anaphase.

Ross, K. E. & Cohen-Fix, O. *Dev. Cell* **6**, 729–735 (2004)

Separase (Esp1 in budding yeast) triggers sister-chromatid separation in anaphase. Interestingly, in the absence of Esp1 function, the undivided nucleus is inherited almost exclusively by the daughter cell. So what causes the daughter-cell preference? These authors show that, independent of its role in sister-chromatid segregation, Esp1 affects nuclear positioning as part of the FEAR (Cdc14 early anaphase release) pathway, which induces a mother-cell-directed pulling force on the spindle poles.

CELL MIGRATION

### Cofilin promotes actin polymerization and defines the direction of cell motility.

Ghosh, M. *et al*. *Science* **304**, 743–746 (2004)

Condeelis and colleagues used an engineered, photoactivatable analogue of cofilin, which is resistant to downregulation by endogenous signalling mechanisms, to study *in vivo* cofilin functions. They found that intracellular cofilin activity causes an increase in F-actin and generates free barbed ends, and it induces local protrusions and determines the direction of cell migration. These findings contradict the previously reported role of cofilin as an actin-depolymerizing factor.

PROTEIN MODIFICATION

### *S*-Nitrosylation of parkin regulates ubiquitination and compromises parkin's protective function.

Chung, K. K. K. *et al*. *Science* 22 April 2004 (doi:10.1126/science.1093891)

Parkin is an E3 ubiquitin ligase that ubiquitylates substrates that are important for dopamine-neuron survival. Mutations in parkin that disrupt its enzymatic activity are the most common cause of hereditary Parkinson's disease (PD). Chung *et al.* now show that parkin is *S*-nitrosylated *in vitro* and *in vivo* in animal models of PD and in the brains of PD patients. *S*-nitrosylation of parkin inhibits its E3 ubiquitin ligase activity and its protective function, and so might contribute to the neurodegeneration process.