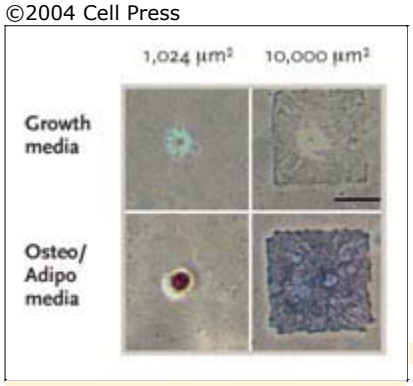
Stem Cell Sculpting

Study notes the power of shape on cell differentiation | By Jack Lucentini



SHAPING A COMMITMENT: Brightfield images of hMSCs plated onto small 1,024 µm^2 or large 10,000 µm^2 fibronectin islands after 1 week in growth or mixed media. Lipids stain red, alkaline phosphatase stains blue. Scale bar = 50 µm. (From R. McBeath et al., *Dev Cell*, 6:483-95, 2004)

Researchers have tried for years to make stem cells differentiate into specific cell types. This work usually involves bathing the cells in molecular signals that affect their fate. Some are finding, though, that cellular shape may be at least as influential as signal molecules in the differentiation process. This adds to a growing literature suggesting that cellular spatial structure affects many activities, including proliferation, apoptosis, and tumorigenesis.

Johns Hopkins University researchers found that shape and related characteristics of human mesenchymal stem cells (bone marrow cells that become fat, bone, cartilage, or muscle) are the strongest known factors determining their fate.[1] The team adopted a technique called micropatterning for modifying cell shape; it involves painting a surface with fibronectin, a cell-attracting protein. Cells cultured in the presence of large fibronectin squares spread out into a flat, nearly pancake-like form as they hug the wide surfaces. Cells cultured with tiny squares become more or less globular, because a cell will attach itself to a small square without spreading. Thus, forcing cells into specific shapes influenced their fate more powerfully than any previously known fate- regulating signals, the researchers say. When these cells were cultured in the same media, says Rowena McBeath, MD/PhD candidate at the university and a coauthor, differentiation into fat cells occurred only on small squares, and bone-cell development occurred only on large ones. Intermediate-sized squares gave rise to both types.

The study did not negate the role of molecular signals; indeed, it

identified a new one, Rho kinase (ROCK), as having influence comparable to that of shape. ROCK increases cytoskeletal tension, which, again, probably has some relationship to shape, the authors say. Shape and ROCK levels now seem to be the two strongest factors determining fate in these types of cells, they add. Either overrides all other known signals, most notably the GTPase Rho, a ROCK-stimulating protein and the strongest previously known signal.

The study "clearly shows that cell shape affects this early developmental fate," says Steven Heidemann, a professor of physiology at Michigan State University. Others previously showed that shape affects fate, "but never as early as at the stem-cell stage," he adds. Earlier findings thus couldn't prove cells had committed themselves to a particular lineage, only that they had upregulated or downregulated certain activities, says Donald Ingber, a professor of pathology at Harvard Medical School and former professor of one of the study's authors.

MURKY DETAILS The new study may also point to mechanisms for this commitment process. It seems a flattened shape stimulates ROCK, which tenses the cytoskeleton's actin fibers via the molecular motor myosin. The tension, or lack of it in balled-up cells, is a decisive factor in whether cells differentiate toward bone or fat respectively, the authors say. Further details are murky, but the study suggests tissue-engineering researchers should consider "what kinds of mechanical environment the cells are sitting in," and which molecular pathways are modulated by shape, says Christopher Chen, senior author and assistant professor of biomedical engineering and oncology at Johns Hopkins.

Mark Pittenger, vice president of research for Osiris Therapeutics, Baltimore, Md., says the findings could aid companies in developing cartilage replacement therapies for arthritis. "Growing cells to appropriate density," as in a part of Chen's study that involved crowding cells to force them into appropriate shapes, could help, says Pittenger.

Others say the findings add to growing evidence that the cytoskeleton serves as a lattice to bring together enzymes for many cellular activities. Gabor Forgacs, University of Missouri professor of biological physics, recently found that cytoskeletal and signaling proteins, such as those implicated in differentiation, associate together more often than any other classes of proteins in yeast.[2]

Disagreement surrounds just how this structure-signal dance works. Ingber espouses a theoretical explanation, called tensegrity, which has attracted both praise and criticism. Tensegrity proposes that the cytoskeleton is a set of tensed and compressed rods akin to structures built by the noted architect Buckminster Fuller. The theory also asserts that tension-induced rearrangements in this lattice affect biochemistry, a claim the latest findings "completely support," Ingber says. Forgacs, a skeptic, insists tensegrity can't explain the speedy movements of real

cytoskeletons, but says either way, "I don't think anyone would seriously argue today that the cytoskeleton is not an important part of intracellular signaling."

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References

1. R. McBeath et al., "Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment," *Dev Cell*, 6:483-95, April 2004.

2. G. Forgacs et al., "Role of the cytoskeleton in signaling networks," *J Cell Sci*, 117:2769-75, June 1, 2004.

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