to limit the expansion of primitive hematopoietic cells and appear to function through similar pathways on this population, highlighting the potentially redundant systems in place to regulate HSC cell-cycle patterns. Notably, genes from this same family are also frequently inactivated in cancerous cells, presumably permitting the continued proliferation of malignant cells. Therefore, the existence of multiple backup mechanisms to prevent dysregulation of stem cell proliferation would appear to be a protective feature in this population. Only through studies such as those described by Viatour et al. (2008) are we beginning to tease apart the complex picture of HSC cell-cycle regulation as dictated by various, likely interconnected, signaling pathways within the cell.

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Patterning Stem Cell Differentiation

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Regulation of cell differentiation and assembly remains a fundamental question in developmental biology. Now, a report from the Chen laboratory (Ruiz and Chen, 2008) describes an approach that represents a major step toward a more profound understanding of the geometric-force control of stem cell differentiation.

In a developing organism, tissues emerge from coordinated sequences of cell renewal, differentiation, and assembly that are orchestrated by spatial and temporal gradients of regulatory factors. Likewise, in an adult organism, tissue regeneration by stem cells—exogenous, or mobilized from the host, or implanted in the form of an engineered graft—depends on the presence, patterns, and timing of multiple factors. The fields of stem cells, tissue engineering, and regenerative medicine are starting to realize just how important the entire context of the cell environment is, with its ever-changing milieu of other cells and three-dimensional matrix and sequences of molecular and physical morphogens. Much is being learned about the molecular and physical control of tissue formation using a range of models that help reveal some of the feedback mechanisms at the cellular, tissue, and organ levels (Lecuit and Le Goff, 2007; Krieg et al., 2008).

Innovative in vitro approaches have been established to probe the effects of mechanical and shape cues on stem cell differentiation. An earlier study from the Chen lab (McBeath et al., 2004) utilized spatial patterning of adhesion molecules to show that a single human mesenchymal stem cell (hMSC) patterned on a small island tends to undergo adipogenic differentiation, while a cell from the same preparation patterned on a larger island (and thereby allowed to spread and develop high cytoskeleton tension) tends to undergo osteogenic differentiation. Lineage commitment could thus be largely regulated by cell shape/size via related changes in cytoskeletal tension. The same lab demonstrated that endothelial cells patterned on a specific geometric shape exhibited position-dependent proliferation profiles (Nelson et al., 2005). For instance, for cells on a ring island, a high proliferation rate was observed at the outer edge, in contrast to a reduced fraction of dividing cells at the inner edge. It was proposed that cell behavior was related to cytoskeleton tension that can be transmitted between neighboring cells through cadherin-mediated connections, such that the individual cells cooperate and are mechanically coupled. In another study (Engler et al., 2006), hMSCs cultured on hydrogels designed to mimic mechanical properties of native tissues showed lineage specification that depended on substrate elasticity, such that soft (brain-like) matrices were neurogenic, somewhat stiffer (muscle-like) matrices were myogenic, and the most rigid (bone-like) matrices were osteogenic. These findings were attributed to the cytoskeleton tension forces that activated molecular pathways of cell differentiation in a manner dependent on substrate stiffness.

Expectedly, tissue engineering is becoming increasingly oriented toward biologically inspired environments for directing stem cell differentiation into hierarchically organized tissues, to help unlock the full potential of stem cells for tissue regeneration (Burdick and Vunjak-Novakovic, 2008). Clearly, the biological complexity of a native developmental context is not mimicked by simple laboratory settings because they lack the interplay of mechanical and molecular factors present in vivo. The field is in need of

a new generation of culture systems that offer something between a Petri dish and a whole-animal model. Such models should be designed to be predictive of normal and pathological situations in vivo, while providing tight control of environmental parameters and offering insight into the cellular responses by way of phenotypic and functional read-outs.

In a recent paper, Ruiz and Chen (2008) describe a nicely designed and rigorously controlled study that opens many doors, answers some of the standing questions, and poses many more. Their technical approach is simple and elegant, representing an extension of their previous work. hMSCs were cultured on adhesive patterns in a range of geometries (circles, squares, rectangles, sinusoidal bands; see Figure 1) and subjected to osteogenic or adipogenic regulatory molecules. The combined effects of regulatory molecules and cell position within the pattern (such as the concave or convex edge of a sinusoidal band) on cell differentiation were evaluated and correlated with traction forces at each location. The gradients of mechanical forces essentially determined the patterning of the two cell lineages, with osteogenesis collocating with the regions of high tension and adipogenesis collocating with the regions of low tension.

The three-dimensional studies, which are most interesting in the context of developmental and adult biology, showed qualitatively the same trend, and it will be important to see if new models will be developed that enable the same level of quantitation shown for two-dimensional systems. A major issue in the extension of this approach into three-dimensional systems is the decoupling of transport and force gradients, each affecting cell function on many levels. Such extension would have major implications on the design of scaffolds for stem cells. It is easy to speculate that we can build scaffolds by printing polymerizable biomaterials with graded mechanical properties and thereby form an instructive template for the formation of a complex tissue structure starting from stem cell populations. We can further speculate that such scaffolds will be capable of mobilizing stem cells from the host and enable clinical modalities with rather simple regulatory paths. Ruiz and Chen (2008) actually demonstrate a "precursor" for such an approach, by the formation of bone around fat, not unlike the structure of native bone.

Other applications of interest include the generation of temporal gradients of regulatory factors, construction of models of disease (for example, stem cell repair of bone or myocardium, tissues known to change their mechanical properties when diseased), and studies of other stem/progenitor cells. The approaches described by Chen and colleagues can be utilized to expand the application of microscale technologies that are becoming powerful tools for stem cell research. Today we can fabricate microscale devices with resolutions as low as several hundred nanometers and use these constructs to control the topography and spatial distribution of molecules and attached cells, while subjecting the cells to physical stimuli (Burdick and Vunjak-Novakovic, 2008). One can expect to combine the best of both worlds—geometry-force control of cells and microscale technologies—into advanced systems for high-throughput screening of stem cells and regulatory factors. As the authors say themselves, gradients in forces and morphogens can help develop a "roadmap" for regenerative therapies.

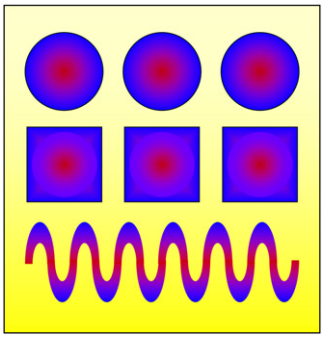

Figure 1. Geometry Determines Spatial Patterning of Differentiation

Human mesenchymal stem cells were formed into aggregates in the shape of a circle (top row), a square (middle row), or a sinusoidal band (bottom row). After 2 weeks of cultivation in culture medium containing the osteogenic and adipogenic supplements, the cells were stained for alkaline phosphatase (blue) and oil droplets (red). The patterns of cell differentiation mirrored the patterns of shape-generated stress such that the regions of higher stress induced osteogenesis, while the regions of low stress induced adipogenesis. The same logic governed spatial patterning in three-dimensional cell structures.

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