Cell Stem Cell
Previews

Microarraying for Mechanosensivitity

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In a recent issue of *Science*, Gilbert et al. combine biomaterial microarrays with lineage tracking to demonstrate that muscle stem cell survival is sensitive to the microenvironment's mechanical stiffness (Gilbert et al., 2010). This work enhances the breadth and depth of knowledge in stem cell mechanobiology.

Stem cells exhibit a number of behaviors—including quiescence, migration, division, death, self-renewal, lineage commitment, and differentiation—that are orchestrated throughout development and into adulthood within multicellular organisms. These behaviors are regulated in large part by stem cell niches, structurally complex microenvironments that present their resident cells with numerous cues in the form of soluble factors, extracellular matrix, and juxtacrine factors from neighboring cells. Although the regulatory importance of biochemical signals within a niche has been extensively investigated and appreciated, it is becoming increasingly recognized that the biophysical and in particular the mechanical properties of the cellular microenvironment are important regulators of cell fate. For example, the stiffness or elastic modulus—the measure of the stress (force per area) required to achieve a given strain (the extent of material deformation) without any permanent deformation—varies over four orders of magnitude in natural tissues from fat to bone, and early landmark work showed that fibroblast migration (Pelham and Wang, 1997) and proliferation and death (Wang et al., 2000) are sensitive to physiologically relevant ranges of substrate stiffness. More recently, Discher and colleagues importantly demonstrated that substrate stiffness could strongly influence the differentiation of mesenchymal stem cells (Engler et al., 2006), behavior later shown also for neural stem cells (Saha et al., 2008) and investigated in greater depth in additional studies.

An important new report makes significant progress in this field by demonstrating that the survival, differentiation, and self-renewal of skeletal muscle stem, or satellite, cells (MuSCs) are regulated by substrate stiffness. Specifically, Gilbert et al. utilized soft lithography to generate an array of laminin-coated microwells fashioned from a polyethylene glycol (PEG) hydrogel, then cultured freshly isolated MuSCs from adult mice on these substrates (Gilbert et al., 2010). Importantly, through use of different polymer precursor concentrations, analogous to studies with polyacrylamide (Pelham and Wang, 1997), the elastic modulus of the PEG network could be varied from softer to harder than muscle while maintaining constant biochemical properties on the substrate surface. Using a newly developed algorithm to track the behavior of single cells within the microwells, the investigators find that cell numbers increase much more rapidly on softer than harder materials, not because of differences in cell proliferation but interestingly because of higher cell death, coupled with higher cell differentiation, on the hard material. Furthermore, cells propagated on soft or stiff materials were implanted into skeletal muscle of immuodeficient mice depleted of endogenous MuSCs and assayed for their capacity to proliferate and engraft over time with bioluminescent imaging. Consistent with numerous prior reports that MuSCs cultured on hard surfaces lose their potency, cells on the stiff hydrogels did not engraft; however, as few as 10–100 cells from gels near the stiffness of muscle could strikingly engraft in vivo. Finally, doublets of cells that had undergone one cell division on the substrates were transplanted into muscle (five doublets per mouse), and doublets from soft but not hard materials were able to engraft. This result argues that a symmetric or asymmetric division can occur on the soft substrates, one or more MuSCs is thereby preserved, and MuSCs can thus undergo self-renewal ex vivo on soft surfaces.

This creative work has a number of important implications. First, in addition to demonstrating mechanosensitivity for another important stem cell, for the first time it does so with acutely isolated stem cells not previously cultured on hard surfaces and by subsequently using an in vivo model to analyze cell potency, thus more effectively establishing the in vivo relevance of the principle. In addition, it can be unclear which stem cell behavior is mechanosensitive; for example, microenvironmental instruction of cellular lineage commitment or selective survival of cells that have adopted a given lineage could give rise to the same endpoint cell culture composition. Although prior technologies have been developed to conduct lineage tracking, this study's application of longitudinal cell tracking to investigate stem cells on variable moduli demonstrates that the mechanical properties of the cellular microenvironment regulated a specific stem cell behavior, in this case cell survival. Finally, although it is not the focus of this work, its results have implications for regenerative medicine. Cell source is a current limitation of muscle stem cell-based therapies, given that MuSCs have been difficult to expand in culture, whereas more committed myoblasts can be expanded yet often progressively lose myodifferentiation potential. This work thus further demonstrates that engineered biomaterials have the potential not only to address fundamental biological questions but potentially also to serve as culture systems to expand and differentiate cells for biomedical applications (Keung et al., 2010).

This study also raises a number of interesting questions. Although focal adhesions and the cytoskeleton are recognized to play a central role in cellular

mechanosensing (Choquet et al., 1997; Pelham and Wang, 1997), the mechanisms by which this information is transduced to impact downstream gene expression and stem cell behavior will be an interesting focus of future work, as indicated in a recent study of angiogenesis (Mammoto et al., 2009). In addition, the lineage analysis in Gilbert et al. indicates that cell survival is a primary mechanosensitive behavior for MuSCs, and future work may elucidate whether the same or different facets of behavior are mechanosensitive in different stem cells. Likewise, the reported microwell system can be applied to investigate whether stem cells from different tissues are sensitive to different stiffness values or "set points," as indicated in work with mesenchymal stem cells (Engler et al., 2006). Furthermore, this study indicated that soft materials support MuSC self-renewal, although it is still conceivable that the MuSCs may have committed to undergo self-renewal prior to being placed on the substrate, indicating that this rich system may continue to have an impact in future studies of longer-term self-renewal and lineage commitment. Finally, Gilbert et al.'s use of primary cells made an important step toward addressing in vivo stem cell mechanosensitivity, and recent investigation of breast tumorigenesis (Levental et al., 2009) may help set the stage for elucidating whether stem cells respond to mechanical properties within their niches in vivo. It is possible for example that both biochemical (Conboy et al., 2003) and mechanical changes in the MuSC niche could underlie the decreased muscle regenerative capacity observed with organismal aging or disease.

In summary, this rigorous study by Gilbert et al. not only increases our knowledge of stem cell mechanobiology but also provides new approaches and avenues to further establish the biological significance and biomedical utility of this expanding field.

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The STATs on Naive iPSC Reprogramming

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Pluripotency can be induced in somatic cells via ectopic expression of defined transcription factors. In this issue of *Cell Stem Cell*, Yang et al. (2010) demonstrate that Lif/Stat3 signaling directly contributes to the in vitro induction of murine naive pluripotency.

The identity of somatic cells can be epigenetically reprogrammed and forced to adapt a new functional cell state by different methods and distinct combinations of exogenous factors. The aspiration to utilize such ex vivo reprogrammed pluripotent and somatic cells for therapeutic purposes necessitates understanding of the mechanisms involved in cellular reprogramming. Takahashi and Yamanaka achieved a major breakthrough by demonstrating that four transcription factors can convert somatic fibroblasts into ESC-like induced pluripotent stem cells (iPSCs) (Takahashi and Yamanaka, 2006). Fully reprogrammed iPSCs share all defining features with embryonic stem cells (ESCs) including gene expression and epigenetic patterns, cell signaling dependence, and developmental potential (Jaenisch and Young, 2008). More recently, it has become apparent that the original Oct4, Sox2, Klf4, and c-Myc combination constitutes a classical recipe for reprogramming and that iPSCs can be derived via extensively modified combinations of transcription factors (e.g., Oct4, Nanog, Klf4, and c-Myc; Nr5a2, Sox2, Klf4, and c-Myc; Oct4, Sox2, Nanog, and Lin28, etc.). After factor transduction, the somatic cells are propagated in vitro and a small fraction of the cells give rise to ESC-like