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Directed stem cell differentiation: the role of physical forces

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Abstract

A number of factors contribute to the control of stem cell fate. In particular, the evidence for how physical forces control the stem cell differentiation program is now accruing. In this review, we discuss the types of physical forces: mechanical forces, cell shape, extracellular matrix geometry/properties, and cell-cell contacts and morphogenic factors, which evidence suggests play a role in influencing stem cell differentiation.

Keywords

Differentiation; mechanical force; morphogenic factors; geometric controls; cell shape; ECM properties; cell-cell contacts

Introduction

The regulation of stem cell differentiation has been a challenge in regenerative medicine. Morphogenesis depends on complex interactions within a dynamic four dimensional environment (three dimensional (3D) plus time). Previous studies suggest that close cell-cell interactions and 3D culture conditions are often necessary prerequisites for differentiation and that cells within 3D culture display distinct features that are more representative of native tissues than are cells within conventional 2D culture (Abbott, 2003; Griffith et al., 2006; Kiger et al., 2001; Radisic et al., 2007; Xie et al., 2000). Tissue engineering seeks to repair or regenerate damaged or diseased tissue and organs through the implantation of combinations of cells, scaffolds, and soluble mediators (Atala, 2008; Guilak et al., 2009; Vacanti et al., 1999). Tissue engineering offers the advantage of a 3D environment as well as flexibility of size and shape and increasingly controllable environmental conditions that can be studied in depth *in vitro*. A detailed understanding of the cellular responses to exogenous stimuli is critical in order to elucidate and therefore control tissue development and remodeling for the generation of optimal tissue engineered grafts.

The decision made by a stem cell to commit to a particular program is highly context dependent and requires multiple targets in different pathways to be simultaneously perturbed to generate a cellular response switching between growth, differentiation, and apoptosis. Stem cells unique advantage of multipotency, the potential for differentiation of multiple cell types, lends them to be a promising cell source for regenerative medicine therapies. However, stem cell multipotency can also lead to unwanted differentiation of an undesired

cell type at an unwanted location or time which may have a detrimental effect on the native physiologic state. To prevent these unwanted responses, stem cells have developed elaborate mechanisms and checkpoints that ensure a differentiation response only when cues are in the appropriate biological context. A number of factors have been shown to play a role in stem cell differentiation including, soluble cues (i.e. growth factors and cytokines), cell-cell contacts, cell-extracellular matrix (ECM) contacts, and physical forces (Figure). Here we briefly review the evidence for biophysical control of stem cell differentiation.

Cell shape and Mechanical forces

Cell shape is determined by various physical forces that include quiescent or resting force (residual strain) and additive mechanical forces including deformational loading and fluid applied forces. Cell shape is a key regulator of many aspects of development and cell physiology (Folkman, 1978) in the myocardium (Manasek et al., 1972) and endothelial cells (Ingber, 1991) as well as others. Changes in cell shape, via mechanical cues, and binding of specific growth factors and ECM proteins to their respective cell surface receptors can switch cells between discrete fates of growth, differentiation, apoptosis, and migration (Dike et al., 1999; Hwang et al., 2006b; Kloxin et al., 2009; Nelson et al., 2005; Sordella et al., 2003). A number of studies have shown that differentiation of adult or embryonic stem cells into a chondrocytic phenotype requires a rounded cell shape (Awad et al., 2004; Erickson et al., 2002; Guilak et al., 2009; Hoben et al., 2008; Hwang et al., 2007; Johnstone et al., 1998; McBride et al., 2008). A cell shape change from round to flattened morphology can profoundly alter the organization of the actin cytoskeleton and the assembly of focal adhesions (Chen et al., 1998, 2003) and reliably switch MSCs between different lineages (i.e., osteoblastic versus adipogenic) (McBeath et al., 2004). Chicurel et al. has shown that when cells are forced to become round, they undergo apoptosis even though they receive growth factor stimulation and remain attached to the ECM, which would normally induce proliferation (Chicurel et al., 1998). Embryonic mesenchymal cells attached to microsurfaces with a diameter less than the cell diameter conserved their original round shape and remained undifferentiated, whereas cells attached to surfaces with diameters larger than the cell diameters became elongated with a shape similar to their *in vivo* counterparts and differentiated (Yang et al., 1999). Thus, one physical parameter, cell distortion, can control the switch between multiple cell fates.

Time-varying changes in mechanical stresses and strains significantly influence the fundamental cellular responses in terms of cell morphology, phenotype, and function of various types of growing tissues; particularly in the skeleton (Burger et al., 1999; Glucksmann, 1942; Takahashi et al., 1996, 2003; Long et al., 1998; Rooney et al., 1992), cartilage (Glucksmann, 1939; Hall, 1967; Hall, 1968), myocardium (Hove et al., 2003), fetal lung epithelium (Liu et al., 2000), kidney (Serluca et al., 2002), and vasculature (Davies, 1995; Orr et al., 2006). Initial work in cardiac tissue engineering studied mechanical conditioning of differentiated native cells (i.e., rat cardiomyocytes and bovine chondrocytes) based on the theory that the forces that determine tissue development and remodeling *in vivo* would also improve tissue development and function *in vitro* (Clause et al., 2009; Tobita et al., 2006; Zimmermann et al., 2000, 2002). Recent work with stem cells has looked at deformational loading (i.e., compressive or tensile) and fluid applied forces (i.e., pressure or shear stress).

Mechanical strain, either cyclic or uniform biaxial, has differential effects on stem cell lineage specificity. Cyclic mechanical stretch has been shown to commit mesenchymal stem cells (MSCs) to a myogenic phenotype in a magnitude and substrate protein-coating dependent manner (Gong et al., 2008; Hamilton et al., 2004; Park et al., 2004; Yang et al., 2000) and to commit mouse embryonic stem cells to a vascular smooth muscle cell

phenotype (Shimizu et al., 2008). Mechanical strain has also been shown to increase proliferation and inhibit differentiation in mouse and human embryonic stem cells (Saha et al., 2006; Shimizu et al., 2008) as well as modulate orientation of cells with respect to the direction of strain (Altman et al., 2002). Cyclic compression has shown to alter MSC phenotype. MSCs subjected to dynamic compression or hydrostatic pressure increases chondrocyte lineage differentiation (evidenced by increased aggrecan, collagen II, and proteoglycans levels) and enhanced extracellular matrix deposition (Angele et al., 2003; Huang et al., 2004; Mauck et al., 2006, 2007; Saitoh et al., 2000). The application of pulsatile or shear flow to MSCs and/or endothelial progenitor cells induces the expression of endothelial cell and smooth muscle cell markers (Gong et al., 2008; Niklason et al., 1999; O'Cearbhaill et al., 2008; Wang et al., 2005; Yamamoto et al., 2003, 2005).

The effect of mechanical forces on stem cell differentiation is dependent upon cell type as well the phenotype/environment it is in. For example, mechanical compression significantly increases the chondrocytic expression of bone-marrow-derived MSCs embedded in a hydrogel; however, embryonic stem cell-derived embryoid bodies significantly downregulate chondrocytic gene expression under the same conditions (Terraciano et al., 2007). Taken together it is clear that mechanical forces, at least in part, regulate stem cell differentiation with the differential effects dependent upon a number of cell specific factors.

Extracellular matrix

The mechanical properties of 3D matrices are emerging as critical regulators of morphogenesis (Adams et al., 1990; Moore et al., 2005), migration (Guo et al., 2006; Pelham et al., 1997), differentiation (Chun et al., 2006; Cohen et al., 2008), apoptosis (Wang et al., 2000), proliferation (Hadjipanayi et al., 2009). The importance of the extracellular matrix (ECM) on stem cell fate has been shown with particular emphasis on the interactions of ECM ligands with cell surface receptors, ECM geometry, or ECM elasticity. ECM has also been shown to be a more potent differentiation cue for MSCs than chemical stimulation (Bennett et al., 2007; Benoit et al., 2008). Engler et al, recently showed that the lineage specification of human MSCs depends on the substrate mechanics: multiple different lineage differentiation can be induced by simply altering substrate compliance in the absence of soluble factors (Engler et al., 2006). Cells are tuned mechanically so that they preferentially differentiate on ECM with a mechanical stiffness similar to that of their natural tissue (Engler et al., 2004; Saha et al., 2008a). Mechanical signals from the elasticity of the ECM may allow the maintenance of MSCs in a quiescent state while preserving their multilineage potential (Winer et al., 2009).

Multiple tissues have similar elasticities; suggesting that definite stem cell differentiation by a single set of mechanical properties (i.e. matrix stiffness at the macroscale level) may not be possible. Topographical patterns, either micro- or nanoscale of the ECM could also be potent regulators of stem cell differentiation (Dalby et al., 2007). High scaffold porosity compared with flat surfaces significantly enhances neurite outgrowth from neurogenically differentiated stem cells (Hayman et al., 2005). Human embryonic stem cells alignment and elongation, through a cytoskeletal-mediated mechanism, is also significantly increased on patterned matrices (Gerecht et al., 2007) a similar response in alignment is also seen with neural stem cells (Recknor et al., 2006). Nanoscale matrix cues are also recognized by cells. Human MSCs align their cytoskeleton and nuclei along nanoscale patterns which increase differentiation markers compared to unpatterned controls (Yim et al., 2007). Neural stem cell differentiation and proliferation responses depend upon fiber diameter (Christopherson et al., 2009). Cellular response can thus be determined by matrix properties at the macro-, micro-, and nanoscale.

Cell-Cell contacts and morphogenic factors

Cell-cell contact and the cellular microenvironment have been shown to play a role in stem cell fate determination, although this remains largely preliminary. Cell-cell contacts, both hetero- and homotypic, have been shown to play a crucial role in development of the cardiovascular system (Arai et al., 1997; Lough et al., 2000) with a similar observation was reported in cardiomyocyte induction from stem cells (Iijima et al., 2003; Rudy-Reil et al., 2004). Cell-cell contact with stromal cells has been shown to induce marked alterations in gene expression in stem cells and to alter their proliferation (Wagner et al., 2005). Tang et al. have also shown that direct cell-cell contact enhances bone marrow-derived stem cell osteogenic and adipogenic differentiation, and the differentiation extend varies with cell-cell contact (Tang et al., 2010). Similarly, a certain minimum density of cells seems to be required for adipogenic differentiation as well as smooth muscle differentiation from cortical stem cells (Parfitt, 1984; Tsai et al., 2000) and that it is an instructive mechanism, rather than selective proliferation and/or survival, that mediated these differences in cell-fate determination.

In addition to direct cell-cell contact, cell-secreted morphogenetic factors can be utilized to modulate differentiation signaling pathways leading to commitment and tissue formation (Hwang et al., 2008). Morphogenetic factors secreted by chondrocytes can regulate MSC chondrogenic and osteogenic differentiation as well as human embryonic stem cell chondrogenic differentiation (Gerstenfeld, et al., 2003; Hwang et al., 2007; Vats et al., 2006). Human embryonic stem cell-derived mesenchymal stem cells can secrete morphogenetic factors that act as paracrine modulators for tissue repair and regeneration in cardiovascular, hematopoietic, and skeletal diseases (Hwang et al., 2008; Sze et al., 2007). These observations together suggest that cell-cell contacts and interactions in the form of secreted morphogenetic factors significantly influence stem cell differentiation.

Conclusion

In addition to the physical factors highlighted in this review, there are many other microenvironmental cues including soluble factors (i.e. growth factors and cytokines) and cell-type and cell-ECM contacts that contribute to cell fate decisions. Similarly, the stimulus-response decisions made by a stem cell can be further complicated by systems biology (Discher et al., 2009): tissue-specific patterns of ligand and receptor expression (Kluger et al., 2004), as well as by sequential autocrine and paracrine inductive loops (Janes et al., 2006) that arise as cell populations develop and adapt (Kirouac et al., 2006). Despite the evidence reviewed here, supporting a key role for physical forces in determining stem cell differentiation responses, little evidence into understanding the underlying mechanisms by which mechanical signals are transduced has been reported. As of yet, no stem-cell specific mechano-sensory mechanisms have been proposed, and any number of mechanisms (i.e. focal adhesions (Balaban et al., 2001; Beningo et al., 2001; Chrzanowska-Wodnicka et al., 1996; Helfman et al., 1999; Ingber, 2006; Riveline et al., 2001; Sniadecki et al., 2007; Tan et al., 2003), changes in membrane curvature or lipid microdomains (Hamill et al., 2001; Rizzo et al., 1998), GPCRs (Chachisvilis et al., 2006), mechanosensitive ion channels (Sukharev et al., 2004), conformational change of cytoskeletal proteins (Johnson et al., 2007; Sawada et al., 2006), the nuclear lamina or nuclear deformations (Lammerding et al., 2004, 2005), and primary cilia (Resnick et al., 2007) may contribute to mechanical control of stem cell differentiation (Cohen et al., 2008). By controlling the mechanical environment of tissue engineered scaffolds, we may further improve the regulation of stem cell fate in artificial systems. The advantages of tissue engineering signify it as an ideal *in vitro* platform to investigate these questions in depth and provide an exciting future direction for stem cell research and tissue engineering.

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Figure 1. Schematic of the factors that have been shown to play a role in stem cell differentiation and the possible cellular responses to those factor.