THE 3D MICROENVIRONMENT AS A REGULATORY CUE DURING MESENCHYMAL STEM CELL OSTEOGENIC DIFFERENTIATION

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ABSTRACT

The bidirectional interaction between cells and their surrounding matrix provides structural and organizational cues for tissue development. It is this intimate interaction that is required for the appropriate and controlled functioning of stem cells within both natural and engineered three dimensional (3D) environments. Elucidation of the mechanisms that govern this interaction are required to guide the induction and maintenance of specific stem cell differentiation in engineered tissues. 3D culture facilitates external mechanical cues, solute concentration gradients and cell-mediated remodeling of the extracellular matrix (ECM) that are required to form many physiologic structures. This work demonstrates that mesenchymal stem cell (MSC) osteogenic differentiation potential is enhanced in 3D type I collagen hydrogel culture as a result of an increase in expression and localization of the discoidin domain receptor 1 (DDR1) that suppresses ERK phosphorylation and enhances type I collagen remodeling. Knockdown of DDR1 results in the reversion of stem cells from a physiologic 3D morphology to a two dimensional (2D) one, thereby inhibiting their ability to upregulate their osteogenic potential in response to the 3D environment. Given its inductive role in MSC differentiation, the ECM can be applied as an instructive cue for differentiating stem cells to promote their ability to regenerate tissue *in vivo*. To demonstrate this application we created defined protein microenvironments using a microbead encapsulation procedure. MSC encapsulated within these environments exhibited osteogenic potential both *in vitro* and *in vivo* and presents a way to engineer the local microenvironment of the stem cell niche for specific lineage commitment. External remodeling and internal signaling, therefore, couple to promote 'tissue' morphogenesis by generating crosstalk between signaling pathways that are geometrically separate in 2D. MSC use DDR1 to sense the three dimensionality of their microenvironment; and changes in cell shape that result from these dynamic matrix adhesions allow cells to integrate their signals in response to local changes in the ECM. MSC responding to injury and the disorganized matrix that they encounter within the wound site must interact through an iterative feed-

back loop to robustly regenerate mature tissue structure and function. Engineering of MSC function will consequently require continued elucidation of biological mechanisms and novel biomaterial development to define local, 3D microenvironments that replicate the control present in native tissue.