3D Shear Stress Microenvironments Alter Embryonic Stem Cell Osteogenic Differentiation through Modulation of CatnB

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Summary: 3D culturing systems, including belly shaker, stirred suspension vessels and automated bioreactors, are compared for their effect on embryonic stem cell differentiation into osteoblasts using gene expression analysis and calcification assays.

Embryonic stem cells (ESCs) are pluripotent stem cells derived from the early embryonic blastocyst. Because of their potential to differentiate into any of the cell types found in the adult body, but also because of their unsurpassed capacity to proliferate in vitro, these cells are a promising resource for cell replacement therapies. Several years ago, we have described the development of an osteogenic differentiation protocol for murine ESCs [1], in which osteogenesis specific genes are stage-dependently expressed when the cells are challenged with ascorbic acid and glycerophosphate and 1α, 25(OH)2 vitamin D3.

A more recent aspect of our work is the transfer of static culture protocols to 3D, which was prompted by the anticipated high demand for clinical supplies of transplantable tissue, which makes it necessary to generate functional bone-specific cells or progenitors in sufficient numbers. Although self-suggesting for large scale stem cell culture, the development of scale-up stem cell protocols in stirred suspension bioreactors has progressed slowly. While the field has made considerable progress with the expansion culture of ESCs and the formation of embryoid bodies in stirred culture systems [2, 3], osteogenic differentiation in 3D has so far not been reported [4].

In this current study, we have compared different 3D culture systems, including belly-shakers and stirred suspension bioreactors that use different vessel sizes and therefore incur various shear forces on the cells, for their ability to support osteogenic differentiation. Assessing differentiation output with quantitative PCR for osteocalcin and matrix calcification (calcium deposition and Alizarin Red S staining) we are able to show that during continuous culture in 3D ESCs shear-dependently down-regulate osteogenic gene expression and fail to calcify.

We have previously described the Wnt signaling pathway as a key pathway that controls the osteogenic differentiation of ESCs in 2D static culture [5]. Based on its key role in the differentiation process, we hypothesized that it may be misregulated in shear conditions causing the failure of the cells to fully mature. The main effector of the Wnt pathway is beta-catenin (CatnB), a protein that is heavily posttranscriptionally regulated. Phosphorylation at certain tyrosine residues will control the localization of this molecule in the cell and therewith its function in either cell adhesion or transcription of target genes. In static osteogenic differentiations, CatnB is typically found in the nucleus when the cells undergo lineage specification, an in vitro differentiation stage that molecularly models in vivo primitive streak formation and gastrulation. Addition of osteogenic inducers, such as vitamin D3 will then reroute CatnB from the nucleus to the plasma membrane [5].

Western blot analysis of CatnB levels proofed that the CatnB wave that is required for proper osteogenic induction in 2D during primitive streak formation was flatter in shear conditions. Our data suggest that misregulation of CatnB nuclear/cytoplasmic ratio during early lineage decisions may cause the inefficient osteogenic differentiation seen with cells that experience shear in stirred suspension cultures.