U.S. FOOD & DRUG ADMINISTRATION

Center for Biologics Evaluation and Research

ADVANCING REGULATORY SCIENCE

Studies of morphological "signatures" might improve characterization of mesenchymal stem cells for use in tissue regeneration

Morphologies adopted by mesenchymal stem cells (MSCs) in the first 3 days of osteogenic induction predicted their ability at day 35 to undergo mineralization

"High Content Imaging of Early Morphological Signatures Predicts Long Term Mineralization Capacity of Human Mesenchymal Stem Cells upon Osteogenic Induction"

Stem Cells

First published online: February 11, 2016

DOI: 10.1002/stem.2322

Ross A. Marklein, Jessica L. Lo Surdo, Ian H. Bellayr, Saniya A. Godil, Raj K. Puri, Steven R. Bauer

Division of Cellular and Gene Therapies, Center for Biologics Evaluation and Research, Office of Cellular, Tissue, and Gene Therapies, US Food and Drug Administration

Establishing universally accepted MSC characteristics that correlate with *in vitro* and *in vivo* outcomes could support the development of these cells for regenerative medicine

- **MSCs are a potential cell source** for a variety of regenerative medicine applications, such as depositing calcium-containing matrices to support bone tissue growth.
- **Current biomarkers** for assessing osteogenic differentiation of MSCs from multiple donor cell sources and passages do not often use measures of multiple osteogenic markers (e.g., gene expression, alkaline phosphatase activity, mineral deposition).
- **Identifying MSC quality attributes** that correlate with *in vitro* osteogenic assay performance could significantly improve identification and screening of cell populations for MSCs with desired properties for bone tissue engineering.
- **One common assay** to determine if MSCs will mineralize (deposit calcium) requires cells to be grown for 35 days in a culture after receiving stimulation, followed by assays for deposition of calcium-containing matrices, an activity associated with bone formation. FDA scientists demonstrated it might be possible to predict if a specific population of MSCs will mineralize by day 3 of stimulation.

Correlation of MSC morphologies with stem cell differentiation potential enabled prediction on day 3 post-stimulation of whether MSCs would mineralize at day 35

- **FDA scientists investigated** whether short term cell and nuclear morphological profiles of MSCs from multiple donors, at multiple passages, correlated with long-term mineralization following osteogenic induction in cell cultures.
- **Automated high content imaging** of early MSC cell and nuclear morphologies showed certain signatures observed 3 days after stimulation were highly correlated with 35 day mineralization and was comparable to other methods of MSC osteogenesis assessment (e.g., alkaline phosphatase activity).
- **The assay used a larger number** of donors than other laboratories that used morphological measures to predict MSC behavior, as well as MSCs from donors of various ages, both genders, and various MSC manufacturing processes.

- MSCs grown in either of two cultures for 3 days
  - osteogenic induction medium
  - non-osteogenic growth medium
- Images of cellular & nuclear shapes of known population of MSCs were analyzed to determine morphology differences between stimulated & non-stimulated MSCs at day 3

Following osteogenic induction, short term cell and nuclear morphological profiles of MSCs from multiple donors (at multiple passages) correlated with long term mineralization.

This imaging approach could be used to compare characteristics of MSC lots from different laboratories and potentially identify morphological signatures that effectively predict their performance in an osteogenesis bioassay.

The methodology could help to significantly improve the ability of scientists to predict which MSCs are suitable for clinical use. Thus, it has the potential to support FDA regulatory efforts to ensure that therapies based on MSCs are safe and effective.