Supporting the Development of Mesenchymal Stem Cells as Medical Therapies

Scientists at the US Food and Drug Administration (FDA) are developing techniques to help identify which mesenchymal stem cells (MSCs) are most likely to be reliable therapies for certain diseases.

MSCs are stem cells that can differentiate—turn into--a variety of cells types, including those that form fat tissue, cartilage, or bones, depending on the conditions of the culture they are grown in. The FDA work is aimed at helping scientists predict whether MSCs have responded to stimuli known to make them differentiate into specific cell types, that is, counting the stem cells that will specifically produce fat, cartilage, or bone.

As a critical part of this work, the FDA scientists, from the Center for Biologics Evaluation and Research (CBER) adapted for the first time an existing laboratory technique called limiting dilution assay to monitor how many MSCs from specific populations of these cells will be able to differentiate into fat-producing cells. As adapted by the CBER researchers, the technique could also be used to identify which MSCs are likely to differentiate into cartilage- and bone-producing cells. (The basic technique is commonly used to determine how frequently any of a variety of types of cells [e.g., immune cells] in a particular population of like cells can perform a certain function.)

The results of the FDA study suggest that the ability MSCs have to differentiate into fat-producing cells depends in part on which donor provided them and on the number of passages the cells undergo. Passaging is the transfer of small numbers of cells from an established culture into a new vessel in order to prevent overcrowding of multiplying cells and start a fresh culture.

MSCs have significant advantages as potential medical treatments for many diseases because they are readily available in bone marrow and adipose tissue (fat), can differentiate into any of a variety of tissues when properly stimulated, and are easily grown in the large numbers needed for use as medical treatments. In addition, MSCs suppress immune system rejection of donated MSCs in those individuals receiving them, increasing the likelihood of treatment success.

However, using MSCs for medical treatments poses two potential problems. First, these stem cells must be passaged repeatedly to ensure there are enough healthy cells available as a treatment for a particular disease. Passaging can reduce the ability of cells to differentiate. Second, since MSCs used to treat patients might come from a variety of donors, they could contain populations of cells that vary in their ability to differentiate, especially after repeated multiplication and passaging. This emphasizes the need to find donor-related factors in MSC variability that affect the clinical usefulness or performance of these cells.

Therefore, the successful adaptation of the limiting dilution assay to MSCs in order to enumerate progenitor cells from different donors and passages that will become effective cells is an important step in support of developing reliable MSC therapy. Specifically, it offers scientists a potential tool for identifying the measurable characteristics that can quickly and reliably predict if a particular population of such cells will proliferate (multiply) and differentiate. It is crucial, therefore, to identify those biological characteristics, such as the expression of specific genes or the presence of specific proteins on the surface of a cell, that are associated with particular biological activities, such as differentiation of MSCs. By using the limiting dilution assay to find those MSCs from different donors and passages that have the potential to differentiate efficiently, scientists can now look for measurable biological characteristics on those successful cells that are specifically linked with that potential. Those characteristics could then be used routinely to identify MSCs likely to produce the type of tissue needed to treat a particular medical condition.

The CBER scientists used a variety of standard laboratory techniques, including the limiting dilution assay, to evaluate the ability of MSCs from two donors (PCBM1632 and PCBM1641) to differentiate

into fat-producing cells after they underwent passaging for the 3rd, 5th, and 7th time. The main finding was that the PCBM1641 population of MSCs maintained its ability to differentiate, while PCBM1632 lost significant potential to do so. Specifically, 1 in 76 PCBM 1641 MSCs retained the ability of the MSCs to reproduce and differentiate into fat-producing cells after all the passages, while only 1 in 2035 PCBM1632 cells did so.

In addition, the larger the cells of either population became, the less likely they were to reproduce and differentiate.

The CBER scientists also measured the expression of genes linked to the differentiation of MSCs into fat-producing cells. Expression of these genes decreased with passage number for MSCs from PCBM1632 and correlated with the decrease in the ability to form fat-producing cells by passage 7. In contrast, MSCs from PCBM1641 showed increased expression of these genes with increasing passage. Such genes might be useful as biomarkers to identify MSCs most likely to become progenitor cells.

Title

"Quantitative Approaches to Detect Donor and Passage Differences in Adipogenic Potential and Clonogenicity in Human Bone Marrow-Derived Mesenchymal Stem Cells"

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