Adipogenic Differentiation and Analysis of MSC

Application Note

Background

Mesenchymal stem cells (MSC) are fibroblastoid multipotent adult stem cells with a high capacity for self-renewal. So far, these cells have been isolated from several human tissues, including bone marrow, adipose tissue, umbilical cord matrix, tendon, lung, and the periosteum [1]. Recently it has been shown that MSC originate from the perivascular niche, a tight network present throughout the vasculature of the whole body. These perivascular cells lack endothelial and hematopoietic markers, e.g. CD31, CD34 and CD45, but express CD146, PDGF-R beta, and alkaline phosphatase [2].

Characterization

According to the position paper published by the International Society for Cellular Therapy (ISCT), MSC express the surface markers CD73, CD90 and CD105 and stain negative for CD14 or CD11b, CD34, CD45, CD79α or CD19, and HLA-DR [3]. In addition to surface marker analysis, the most common and reliable way to identify a population of MSC is to verify their multipotency. MSC can differentiate into adipocytes, osteoblasts, myocytes, and chondrocytes in vivo and in vitro [1,4]. Trans-differentiation of MSC into cells of non-mesenchymal origin, such as hepatocytes, neurons and pancreatic islet cells, has also been observed in vitro when specific culture conditions and stimuli are applied [1]. The directed differentiation of MSC is carried out in vitro using appropriate differentiation media, such as the ready-to-use PromoCell MSC Differentiation Media (see below for differentiation protocol). Terminally differentiated cells are histochemically stained to determine their respective identities (see below for staining protocol).

Marker

- CD146+
- PDGF Receptor-β+
- Alkaline Phosphatase+
- CD31−
- CD34−
- CD73+
- CD14+ or CD11b−
- CD90+
- CD34−
- CD105+
- CD45−
- CD19+ or CD79α−
- HLA-DR−
Adipogenic Differentiation

1. Coat the culture vessel
   Coat a 6-well tissue culture plate with 10 µg/ml human or bovine fibronectin (C-43060/C-43050) according to the instruction manual.

2. Seed the Mesenchymal Stem Cells
   In a fibronectin-coated 6-well tissue culture plate, plate $1 \times 10^5$ MSC per well using MSC Growth Medium 2 (C-28009). Work in duplicate.

3. Let the Mesenchymal Stem Cells grow
   Allow the cells to reach 80–90% confluency. This will take 24–48 hours.

4. Induce the Mesenchymal Stem Cells
   Induce one of the duplicate samples with MSC Adipogenic Differentiation Medium 2 (C-28016). Use MSC Growth Medium 2 for the remaining well as a negative control.

5. Differentiation of the induced Mesenchymal Stem Cells
   Incubate for 12–14 days. Change the medium every third day taking care not to disturb the cell monolayer.

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**Fig. 1:** Lipid vesicle accumulation in adipocytes differentiated from hMSC-BM (human MSC derived from bone marrow) using the PromoCell MSC Adipogenic Differentiation Medium 2 (C-28016). The differentiated culture exhibits extensive intracellular lipid vacuole formation typical of mature adipocytes (left: 40x magnification; right: 100x magnification).

**Fig. 2:** Sudan III staining of intracellular lipids in hMSC-BM-derived mature adipocytes. The cells were cultured for 12 days in PromoCell MSC Growth Medium 2 (C-28009) as a negative control (left) or the MSC Adipogenic Differentiation Medium 2 (C-28016) for the differentiation sample (right). In contrast to the negative control, the mature adipocytes differentiated from MSC exhibit intracellular lipid vesicles (bright red staining).
Important: Do not let the cells dry for longer than 30 sec. throughout the entire staining procedure!

Adipocyte Detection

In mature adipocytes intracellular lipid vesicles are typically observed in large numbers (Fig. 1). These can be highlighted using a lipophilic dye, such as Sudan III, which stains lipid accumulations bright red (Fig. 2).

1. Prepare solutions and buffers
   Use Saccomanno Fixation Solution (Morphisto, #13881.00250) and Sudan III Solution (Morphisto #10396.00500). Prepare a 60% isopropanol solution with distilled water.

2. Wash the cells
   Remove the cells from the incubator and carefully aspirate the medium. Gently wash the cells with Dulbecco’s phosphate-buffered saline (PBS) w/o Ca\(^{++}\)/Mg\(^{++}\) (Cat. No. C-40232).
   **Note:** Do not disrupt the cell monolayer!

3. Fixation of the cells
   Carefully aspirate the PBS and add enough Saccomanno Fixation Solution to cover the cell monolayer. Incubate at room temperature for at least 30 min.

4. Dilute the staining solution
   During fixation, dilute 10 ml Sudan III Solution with 1.5 ml distilled water and pass through a syringe filter. Use within 30 min.

5. Wash the cells
   Carefully aspirate the fixation buffer and wash the cell monolayer with distilled water. Gently aspirate the water and add enough 60% isopropanol to cover the cell monolayer. Incubate at room temperature for 5 min.

6. Add the staining solution
   Carefully aspirate the 60% isopropanol and add enough diluted Sudan III staining solution to cover the cell monolayer. Incubate at room temperature for 10–15 min.

7. Wash the cells
   Carefully aspirate the staining solution and wash the cell monolayer several times with distilled water until the water is clear. Blot the vessel containing the stained cells upside down on a paper towel to remove as much water as possible.

8. Analyze the cells
   Cover with PBS and analyse the stained samples promptly as the dye tends to fade upon prolonged light exposure. Intracellular lipid vesicles in mature adipocytes will be stained bright red (see Fig. 2).
References


Related Products

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