Induced differentiation of macaque adipose-derived stem cells in vitro

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To the Editor: Bone marrow mesenchymal stem cells (BMSCs) are the most studied adult stem cells. However, the deficiencies of BMSCs still remain, such as their low purification rate, limited number, and trauma to the human body. In recent years, some studies have demonstrated that some cells in the adipose tissue extracts bear a similar function to BMSCs, the potential to differentiate into various tissues, known as adipose-derived stem cells (ADSCs). Compared with BMSCs, ADSCs possess the advantages of a wider range of sources, easier collection, less pain for patients, rapid proliferation, no immune rejection, and no moral and ethical issues involved. At present, the studies on ADSCs are mostly limited to lower mammals, such as rabbits and mice, and the experimental research reports on primates are less. Primates have similar anatomical and physiological features to humans, which has become a popular option for many animal experiments. The purpose of this study was to explore the isolation method and potential multipotent differentiation of ADSCs derived from macaques in vitro, providing the experimental data and new insights for primate ADSCs in regenerative medicine.

This study was approved and supervised by the animal ethics committee of the Second People’s Hospital of Yunnan Province. The treatment of animals in all experiments conforms to the ethical standards of experimental animals. According to the methods mentioned by the previous study, ADSCs were extracted from abdominal subcutaneous adipose tissue of macaque (Kunming Primate Research Center, Chinese Academy of Sciences) under aseptic conditions. The cell morphology, growth, and cell cycle status of ADSCs were observed and recorded under the inverted microscope (DS-Ri2, Nikon, Japan). The cell growth was detected by cell counting kit-8 (CCK-8; Beyotime Biotechnology, Shanghai, China) assay. Oil red O staining (Solarbio, Beijing, China) was applied to visualize fat. The cell cycle was detected by flow cytometry (Accuri C6 Plus, BD, US). Besides, the cells were cultured in chondrogenic induction medium (Dulbecco modified Eagle medium/nutrient mixture F-12 [DMEM/F12; Gibco, Shanghai, China] containing 10% fetal bovine serum [FBS, Gibco, Australia], 6.25 mg/L insulin [Thermo Scientific, Shanghai, China], 10 μg/L transforming growth factor-β [Thermo Scientific], and 50 nmol/L vitamin C [Thermo Scientific]), osteogenic induction medium (DMEM/F12 containing 10% FBS, 0.1 μmol/L dexamethasone [Sigma-Aldrich, Darmstadt, Germany], 50 μmol/L ascorbic acid [Sigma-Aldrich], and 10 mmol/L β-glycerophosphate sodium [Sigma-Aldrich]) and adipogenic induction medium (DMEM/F12 containing 10% FBS, 0.5 mmol/L 3-isobutyl-1-methylxanthine [Sigma-Aldrich], 1 μmol/L dexamethasone, 10 μmol/L insulin, and 200 μmol/L indomethacin [Sigma-Aldrich]) to verify the multipotent differentiation of ADSCs. Different staining methods (chondrogenic induction: toluidine blue [Sigma-Aldrich]; osteogenic induction: Von Kossa [Solarbio]; and adipogenic inductions: Oil red O) were used to confirm the cell types.

ADSCs morphology was observed under a microscope. The primary cells began to adhere to the wall 2 to 3 h after seeded into the culture flask. Initially, they were round or elliptical shapes of different sizes. After 12 h of incubation, part of the cells began to stretch into short spindle shapes and triangles. Another 12 h later, most of the cells adhered to the wall. ADSCs grew slowly in the first 3 to 4 days, and then the growth rate began to accelerate. The number of fusiform-shaped fibrous cells was increased significantly. The cells gradually touched each other and covered the whole bottom of the culture flask, and most of the cells exhibited triangular or long fusiform shapes, with increasing the cell volumes and growth rate. The cell morphology remained basically unchanged and kept proliferating vigorously after three consecutive cell passages.

The results of the CCK-8 assay showed that the ADSCs’ proliferation capacity had undergone transitions from the
slow growth rate phase to the rapid growth rate phase. The growth rate on day 4 was nearly twice as fast as day 2 [Figure 1A]. The cell cycle of ADSCs at passage 3 was checked using flow cytometry. The results represented that G1 phase cells accounted for the vast majority, reaching up to 75.1%, and the S phase cells accounted for 5.42% [Figure 1B]. These results indicated that ADSCs were in an active state and transferred to the proliferative phase.

The chondrogenic induction results of ADSCs represented that the growth rate of ADSCs began to accelerate on days 5 to 6 after chondrogenic induction. The morphology of ADSCs started to transfer from fusiform shape to oval or irregular shape, and the volume and size of cells began to increase on day 7. Subsequently, the surface of aggregated cells was covered by the matrix secreted by the cells, and the boundary of the cells became blurry. The cell morphology on day 21 was shown in Figure 1C–1E. The toluidine blue-positive cells at week 3 were shown in Figure 1F. The osteogenic induction images showed the morphology of ADSCs started to transfer from fusiform shape to cubic, polygonal, or irregular shape, and the volume size of cells began to increase on day 7. Subsequently, the surface of aggregated cells was covered by the matrix secreted by the cells. Calcified nodules could be observed on day 13 to 14, and the cell structure could not be seen clearly at this time. The cell morphology on day 21 was shown in Figure 1G–1I. Von Kossa staining was performed at week 3, and the images presented the Von Kossa-positive cells [Figure 1J]. In addition, after adipogenic induction, the morphology of ADSCs changed from fusiform shape to round or irregular shape on day 10, and the lipid droplets were widely distributed in many cells on day 14. With the extension of induction time, the lipid droplets became larger, and the results of adipogenic induction at week 3 were shown in Figure 1K–1M. Oil red O staining was performed at week 3. As shown in Figure 1N, numbers of red-stained regions appeared in the cytoplasm at week 3. The staining results indicated the potential adipogenic capacity of ADSCs.

ADSCs exist in the capillaries and adventitia of large blood vessels of adipose tissues, sharing common morphology and differentiation potential characteristics with BMSCs. At present, most ADSCs are derived from small animals in regenerative medicine experiments, while few studies are based on primates. The physiological and anatomical structures of primates are closest to humans, as well as the experimental results. To promote its application in tissue engineering and regenerative medicine research, further investigations involving the biological characteristics and multipotent differentiation potential of macaque ADSCs should be prioritized.

In the current study, we collected macaque ADSCs from its abdominal subcutaneous adipose tissues using collagencase and neutral protease digestion. The isolated ADSCs showed fusiform- and whirlpool-shaped structures. ADSCs grew stably and quickly, and the morphology and growth rate of cells showed little changes under the microscope. Some researchers demonstrated that macaque ADSCs can grow continuously and rapidly, which can retain stable characteristics at the time of passaging, even to 20 to 30 passages. The chondrogenic, osteogenic, and adipogenic inductions were performed to verify the characteristics of ADSCs. Different staining methods were used to confirm cell types.
In this study, we investigated the multipotent differentiation capability of ADSCs derived from macaque abdominal subcutaneous adipose tissues, demonstrating that ADSCs could differentiate into chondrocytes, osteoblasts, and adipocytes. In recent years, the studies on ADSCs are mainly focused on animal experiments in vitro or in vivo, but there are still many aspects to be determined in clinical practice. The application of ADSCs in regenerative medicine needs further research.

**Funding**

This work was supported by the Yunnan Provincial Science and Technology Department Kunming Medical University Joint Special Project (No. 2019FE001 (-170))

**Conflicts of interest**

None.

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**How to cite this article:** Jiang JL, Li T, Bi X, Wu ZX, Hou KY, Chen Z. Induced differentiation of macaque adipose-derived stem cells in vitro. Chin Med J 2021;134:2379–2381. doi: 10.1097/CM9.000000000001486