Adipose-derived marrow stromal cells from liposapprase are CD271-positive

Kathrin Godthardt*, Prof. Anton Wernig†, Dr. Jürgen Schmitz‡

† Miltenyi Biotec GmbH, R&D Department, Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany | ‡ University Bonn, Department of Physiology, Wilhelmstrasse 31, 53111 Bonn, Germany

*Correspondence to: Kathrin@miltenyibiotec.de; phone +49 (0)2204-8306-4135; fax +49 (0)2204-8306-4890

Introduction

Marrow stromal cells (MSCs) are coming more and more into focus for a variety of clinical research applications, such as for the reduction of graft-versus-host disease (GVHD) after allogeneic transplantation1, or the treatment of osteogenesis imperfecta2 and metabolic diseases3. Bone marrow has long been known to be a rich source for MSCs. However, over the last few years, alternative sources in humans have also been shown to contain MSC-like cells, such as adipose tissue (AT); placenta4, and umbilical cord blood. AT comprises a source of stem cells that can be obtained under less invasive conditions for the patient than bone marrow aspiration. It also shows higher colony forming unit fibroblasts (CFU-F) activity compared to bone marrow as well as the ability to differentiate into cells of the mesenchymal lineage in vitro5, in the form of cosmetic liposapprase, comprises a heterogeneous cell mixture composed of, among others, endothelial cells, hematopoietic cells, smooth muscle cells, pericytes, and cells of mesenchymal origin6. These stem cells are referred to as adipose-derived mesenchymal stem cells (AMSCs). Several researchers have shown that MSCS from bone marrow can be enriched very effectively by selection for CD71 expression7 and have already been used successfully in animal studies8. It is also thought that AMSCs are CD271-positive9. Here, we demonstrate that CD271 can be used to highly enrich AMSCs from liposapprase.

Methods

The aspirate was processed using a standard protocol (Fig. 1). The stromal vascular fraction (SVF) was analyzed by flow cytometry using the following antibodies: CD14, CD34, CD45, CD105, CD146, and CD271 (LNGFR). CD271+AMSCs were enriched by MACS® Technology and expanded using NH Expansion Medium. Therefor, 2×10⁵ cells of the SVF were separated using the CD271 (LNGFR) MicroBead Kit (APC) by magnetic labeling of the cells with CD271 (LNGFR)-APC and Anti-APC Microbeads. After separation, the negative and positive fractions were cultivated in NH Expansion Medium in comparison to AMSCs, isolated by plastic adherence (PA) using the same cultivation conditions. To assess clonogenic potential, a colony-forming unit fibroblast (CFU-F) assay was performed. For this assay, 1×10⁴ cells, respectively, of the SVF, of the negative fraction, as well as a sample of the CD271-positive fraction were cultivated in NH Expansion Medium for 14 days. AMSCs, isolated by MACS Technology according to CD271 expression or by PA, were also expanded for 7 passages with NH Expansion Medium. After expansion, the expression profile was investigated using flow cytometry. Furthermore, the multipotent differentiation potential was determined by cultivating the AMSCs in NH AdipoDiff Medium and NH OsteoDiff Medium. AMSCs cultured in NH Expansion Medium served as negative control.

Results

1. Processing of liposapprase using a standardized protocol

2. Phenotyping of CD271-positive cells of the stromal vascular fraction (SVF)

3. Positive selection of CD271-positive cells from SVF of processed liposapprase

4. Colony-forming unit fibroblast (CFU-F) assay

5. Flow cytometric analysis of ex vivo-expanded AMSCs isolated by CD271 separation and plastic adherence

6. Differentiation potential of AMSCs isolated by CD271 separation vs. plastic adherence

Conclusion

As already described, MSCs from bone marrow, AMSCs from adipose tissue can also be highly enriched according to the expression of CD271 using MACS® Technology. The isolated AMSCs maintain the ability to differentiate to cells of adipocytic and osteoblast lineages.

References