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

Kathrin Godthardt[1]*, Prof. Anton Wernig[2], Dr. Jürgen Schmitz[1]

[1] Miltenyi Biotec GmbH, R&D Department, Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany | [2] University Bonn, Department of Physiology, Wilhelmstraße 31, 53111 Bonn, Germany

*Correspondence to: KathrinG@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 transplantation[1], or the treatment of osteogenesis imperfecta[2] and metabolic diseases[3]. 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)[4], placenta[5], 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 a 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 vitro*[6]. AT, in the form of cosmetic lipoaspirate, comprises a heterogeneous cell mixture composed of, among others, endothelial cells, hematopoietic cells, smooth muscle cells, pericytes, and cells of mesenchymal origin[7]. 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 CD271 expression[8,9] and have already been used successfully in animal studies[10]. It is also thought that AMSCs are CD271-positive[11]. Here, we demonstrate that CD271 can be used to highly enrich AMSCs from lipoaspirate.

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, CD90, CD105, CD146, and CD271 (LNGFR). CD271$^+$ AMSCs were enriched by MACS® Technology and expanded using NH Expansion Medium. Therefor, 2×10^7 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, 1×10^4 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.

Conclusion

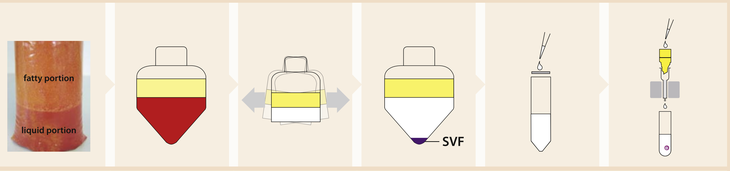
As already described for MSCs from bone marrow, AMSCs from adipose tissue can also be highly enriched according to the expression of CD271 using MACS® Technology. The isolated AMSCs maintain the ability to differentiate to cells of adipocyte and osteoblast lineages.

References

1. Le Blanc *et al.* (2004) Lancet 363: 1439–1441.
2. Horwitz *et al.* (2001) Blood 1; 97:1227–1231.
3. Koc *et al.* (2000) J. Clin. Oncol. 18: 307–316.
4. Zuck *et al.* (2002) Mol. Biol. Cell. 13: 4279–4295.
5. Battula *et al.* (2007) Differentiation 75: 279–291.
6. Bieback *et al.* (2006) Stem Cell Express 24: 1294–1301.
7. Zuck *et al.* (2001) Tissue Eng. 7: 211–228.
8. Quirici *et al.* (2002) Exp. Hematol. 30: 783–791.
9. Jones *et al.* (2004) Blood 104: ASH Abstract 2337.
10. Mifune *et al.* (2007) 53rd Annual Meeting of the Orthopaedic Research Society Poster No: 0947.
11. Meyerrose *et al.* (2007) Stem Cells 25: 220–227.
12. Dominici *et al.* (2006) Cytotherapy 8: 315–317.

Results

1 Processing of lipoaspirate using a standardized protocol



1.	2.	3.	4.	5.	6.
Lipoaspirate not older than 24 hours was used. Optimal results can be achieved with material, which was obtained by tumescence liposuction.	The fatty portion was diluted 1:1 with PBS and centrifuged to exclude all hematopoietic cells. This washing step of the fatty portion was performed two times.	To achieve dissociation of the fatty portion, 0.3 U/mL collagenase was used and incubated for 30 min and 37 °C on an orbital shaker (250 rpm).	The digestion was stopped with the same volume of DMEM/FCS, centrifuged down with 600×*g* for 10 min and re-suspended in buffer.	The stromal vascular fraction (SVF) was filtrated with a 100 µm and a 40 µm cell strainer to exclude cell aggregates and endothelial cells.	After using a MACS® Pre-Separation Filter, AMSCs can be easily isolated by MACS® Technology.

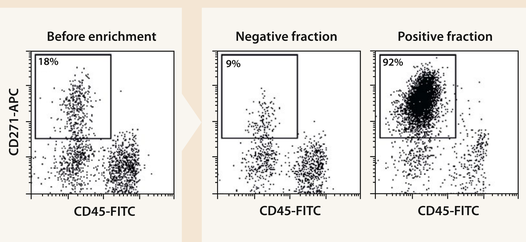
2 Phenotyping of CD271-positive cells of the stromal vascular fraction (SVF)

C

	Gated of all CD271-positive cells (R1)
CD45	4%
CD14	5%
CD90	18%
CD34	53%
CD105	58%
CD146	74%

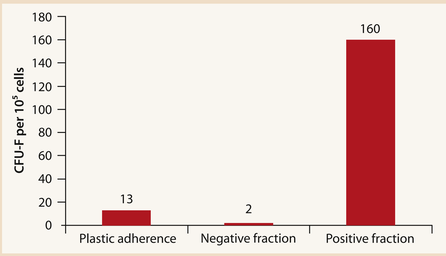
To investigate the phenotype of CD271-positive cells of the SVF, cells were labeled with CD271 (LNGFR)-APC in combination with CD14, CD34, CD45, CD90, CD105, or CD146. The dot plot (A), as an example, shows all viable cells of the SVF labeled with CD271 and CD105. R1, which comprises all CD271-positive cells, is shown in the histogram (B) to detect the frequency of all CD105 double-positive cells (—) compared to the isotype control (—). The results (C) show the corresponding frequencies. Some MSC markers like CD90, CD105, CD146 are partially expressed on CD271-positive cells.

3 Positive selection of CD271-positive cells from SVF of processed lipoaspirate



2×10^7 cells of the SVF were isolated using CD271 (LNGFR)-APC and Anti-APC MicroBeads. Samples of all fractions were labeled with CD45-FITC. PI immunofluorescence and light scatter signals were used to gate on viable cells. The positive fraction contained CD271-positive cells with a purity of 92% (average 82% ± 10%; n=4).

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

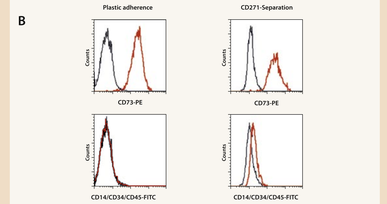


All fractions were Giemsa stained (data not shown) and CFU-F numbers were counted after 14 days of incubation. The number of CFU-F is more than 10-fold higher when AMSCs were isolated according to CD271 as compared to PA.

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

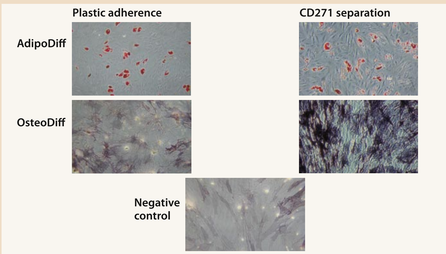
A

AMSC Fraction	CD73	CD105	CD271	CD14	CD34	CD45
PA	+	+	–	–	–	–
CD271-positive fraction	+	+	–	–	–	–



After cultivation of AMSCs for 7 passages in NH Expansion Medium, cells were trypsinized and stained with antibody conjugates for different markers (—) and the corresponding isotype controls (—). *In vitro*–expanded MSCs express CD73, CD105 and lack expression of CD14, CD34, and CD45[12]. Independent on whether AMSCs were isolated by PA or according to CD271 expression, the cells matched these criteria after expansion. Furthermore, CD271 expression is down-regulated during culture (A). The histograms (B) show, as an example, CD73 and CD14/CD34/CD45 staining for both samples.

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



After 18 days of cultivation in NH AdipoDiff Medium, all AMSCs showed an increased accumulation of intracellular lipid vacuoles, as revealed by oil-red-O staining. After 10 days of cultivation in NH OsteoDiff Medium, all MSCs showed a high alkaline phosphatase (AP) activity. Detection of AP activity was performed with Fast BCIP/NBT substrate. Negative controls showed no differentiation.