To the editor:

Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation

Human mesenchymal stem cells (MSCs) are multipotent stem cells, able to differentiate into multiple mesenchymal lineages.[1-3] Previously, we have shown that human fetal lung–derived MSCs enhance the engraftment of human umbilical cord blood (UCB)–derived $CD34^+$ hematopoietic cells in nonobese diabetic–severe combined immunodeficiency mice.[1] Here we show that second-trimester amniotic fluid is an abundant source of fetal MSCs that exhibit a phenotype and multilineage differentiation potential similar to that of postnatal bone marrow (BM)–derived MSCs. We suggest that amniotic fluid is an attractive source of MSCs for cotransplantation in conjunction with UCB-derived hematopoietic stem cells.

Amniotic fluid was collected transcervically from 6 second-trimester legal terminations of pregnancy (mean gestational age, 19 weeks [range, 17-22 weeks]) according to a protocol approved by the medical ethical review board of our hospital. Amniotic fluid samples, without visible contamination with blood, were centrifuged for 10 minutes at 1283 rpm. Pellets were resuspended and cultured as described previously.[1] Adherent cells were detached with trypsin/EDTA (ethylenediaminetetraacetic acid) and phenotypically characterized by flow cytometry using fluorescein isothiocyanate– or phycoerythrin-conjugated antibodies. The adipogenic and osteogenic differentiation capacity of culture-expanded MSCs was determined as previously reported.[1] To confirm the fetal origin of cultured cells, a molecular HLA typing was performed on DNA obtained from expanded MSCs, and fetal and maternal blood cells by polymerase chain reaction/sequence-specific oligonucleotide using a reverse dot blot method.[4]

MSCs were cultured from all 6 consecutive samples of second-trimester amniotic fluid. A quantity of 2 mL amniotic fluid was sufficient to culture these cells. The expansion potential of amniotic fluid–derived MSCs exceeded that of BM-derived MSCs. As a result, we were able to expand amniotic fluid MSCs to about 180×10^6 cells within 4 weeks (3 passages). The phenotype of the culture-expanded amniotic fluid–derived cells was similar to that reported for MSCs derived from second-trimester fetal tissues and adult BM[1,2] (Table 1). Amniotic fluid–derived MSCs showed multilineage differentiation potential into fibroblasts, adipocytes, and osteocytes. Molecular HLA typing of fetal and maternal cells confirmed that the cultured cells were of fetal origin, without detectable contamination of maternal cells (Figure 1).

Following allogeneic transplantation, most studies indicate that MSCs remain of host origin,[5] possibly as a result of the low frequency of these cells in stem cell grafts. The frequency of MSCs in UCB is particularly low, and most laboratories have been unable to grow MSCs from UCB.[6,7] Supplementing stem cell grafts with MSCs to promote engraftment has been proposed. Studies in mice and sheep show that engraftment can be promoted by the addition of third-party MSCs.[1,8] Preliminary clinical studies support these data and suggest that cotransplantation with MSCs derived from the stem cell donor also results in a reduced incidence of graft-versus-host disease.[9]

Table 1. Immunophenotype of culture-expanded second-trimester MSCs: expression of antigens on cultured MSC-derived from amniotic fluid

Antigen	CD no.	Expression
Mesenchymal		
Thy-1	CD90[d]	+
Endoglin, SH2	CD105[e]	+
SH3, SH4[a]	CD73	+
SB10/ALCAM	CD166[f]	+
HLA		
HLA ABC[b]	None	+
HLA DR[c]	None	−
Integrins		
VLA-4	CD49d[g]	−
VLA-5	CD49e[g]	+
LFA-1	CD11a[c]	−
Selectins		
E-selectins	CD62E[h]	−
L-selectins	CD62L[h]	−
P-selectins	CD62P[h]	−
Hematopoietic		
LCA	CD45[c]	−
gp105-120	CD34[c]	−
LPS-R	CD14[c]	−
Ig superfamily		
PECAM	CD31[i]	−
LFA-3	CD58[j]	±
ICAM-1	CD54[f]	+
ICAM-3	CD50[d]	−
VCAM-1	CD106[k]	−
B7.1	CD80[g]	−
B7.2	CD86[d]	−
Cytokine receptors		
IL-2 receptor	CD25[c]	−
IL-3 receptor	CD123[d]	±
IL-7 receptor	CD127[g]	−
TNF-α1 receptor	CD120a[k]	−
TNF-α2 receptor	CD120b[k]	−
Other antigens		
Transferrin receptor	CD71[f]	±
HCAM-1	CD44[d]	+
Bp50	CD40[l]	−

+ indicates positive; −, negative; VLA, very late antigen; LFA, leukocyte function antigen; LCA, leukocyte common antigen; LPS-R, lipopolysacharid-receptor; PECAM, platelet-endothelial cell adhesion molecule; ±, weakly positive; VCAM-1, vascular cell endothelial molecule; and HCAM-1, homing cellular adhesion molecule.

[a]A gift of Dr. A. Moseley, Osiris Therapies, Baltimore, MD.
[b]Instruchemie, Hilversum, the Netherlands.
[c]Beckton Dickinson, San Jose, CA.
[d]PharMingen, San Diego, CA.
[e]Ancell, Bayport, MN.
[f]CLB, Amsterdam, the Netherlands.
[g]Immunotech Coulter, Marseille, France.
[h]Bio-Whittaker, Verviers, Belgium.
[i]DAKO, Glostrup, Denmark.
[j]Southern Biotechnology Associates, Birmingham, AL.
[k]R&D Systems, Abingdon, United Kingdom.
[l]Serotec, Oxford, United Kingdom.

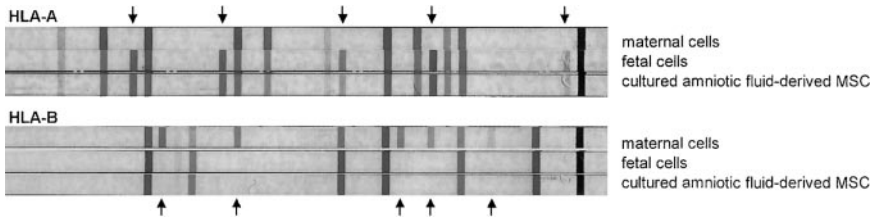

Figure 1. The Dynal Reli SSO (Dynal Biotech, Hamburg, Germany) reverse line blot strip assay was used for molecular typing of the HLA-A and HLA-B locus alleles of maternal cells, fetal cells, and culture-expanded amniotic fluid–derived MSCs from the same sample. The HLA-A and HLA-B type of the culture-expanded amniotic fluid–derived MSCs is identical to the fetal HLA-A and HLA-B type and mismatched with the maternal HLA-A and HLA-B type. Upward arrows indicate maternal specific HLA antigens, and downward arrows indicate fetal-specific HLA antigens.

Cotransplantation of UCB and haploidentical MSCs derived from parental BM is proposed as a strategy to reduce the delay in engraftment that is associated with UCB transplantation. The presence of MSCs in second-trimester amniotic fluid enables the possibility of cotransplantation of hematopoietic stem cells and MSCs from the same donor. This could be particularly useful in the setting of UCB transplantation between siblings.

Pieternella S. in 't Anker, Sicco A. Scherjon, Carin Kleijburg-van der Keur, Willy A. Noort, Frans H. J. Claas, Roelof Willemze, Willem E. Fibbe, and Humphrey H. H. Kanhai

Correspondence: W. E. Fibbe, Department of Hematology, C2R, PO Box 9600, 2333 AA Leiden, The Netherlands; e-mail: fibbe.hematology@lumc.nl

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To the editor:

In B-cell chronic lymphocytic leukemias, 7q21 translocations lead to overexpression of the *CDK6* gene

Abnormalities of chromosome 7q have rarely been reported in chronic B-cell lymphoproliferative disorders.[1] Chromosomal translocation leading to overexpression of the *CDK6* gene, which is located in 7q21, has recently been described in splenic marginal zone lymphoma (SMZL).[2,3] CDK6 together with CDK4 are serine/threonine kinases that positively regulate the G1 phase progression in association with D-type cyclin. Overexpression of CDK6 is considered to contribute to leukemogenesis through dysregulation of cell proliferation, thereby favoring progression through the G1 phase. It seems likely that CDK6 overexpression is implicated in lymphomas with low proliferative index and, as we show here, may also be involved in other low-grade malignancies such as B-cell chronic lymphocytic leukemia (B-CLL). We have previously described the molecular characterization of a t(7;14)(q21;q32)

Table 1. Clinical and molecular characteristics of B-CLL patients with the CDK6 rearrangement

Patient	Sex/age at diagnosis, y	Overall survival (y of follow-up)	Karyotype	FISH analysis		SB analysis
				CDK6	Ig	
1	M/61	Alive (6)	46,XY,t(7;22)(q22;q11), del(13)(q13q14)[12]/46,XY[9]	Split	Igλ split	R
2	M/79	Alive (3)	46,X,−Y,+12[1]/46,X,−Y,+12, t(7;14)(q21;q32)[16]/46,XY[3]	Split	IgH split	NA
3	M/61	Died (16)	45,XY,t(2;7)(p11-12;q22),der(8) t(8;17)(p12;p11), −17[5]/47,XY, t(2;7),+5,+12[10]/46,XY[35]	Split	NA	R

FISH analysis was sequentially performed on the same mitosis with mixed Human BAC Clone GS119p5 and Clone GSI-552A1 (encompassing intron 1, exon 1, and 178 kbp upstream of the coding sequence of the *CDK6* gene; Incyte Genomics, San Francisco, CA) and with IgH probes (Vysis, Downers Grove, IL) or Igλ probes (kindly provided by Reiner Siebert). The *CDK6* gene was split between chromosomes 7 and 22 in FIO, between chromosomes 7 and 14 in DUP, and between chromosomes 2 and 7 in LAN. Igλ and IgH involvement was further demonstrated in patients 1 and 2, respectively. It may be noticed that the CDK6 GS119p5 probe gave a slight cross-hybridization with 3q chromosome. Southern blot analyses were performed with WS2 probe[4] and with *CDK6* PCR-amplified cDNA probes: E1 (nucleotides 12 to 298; GenBank accession no. X66365), p123, and p45 (nucleotides 446 to 860 and 61817 to 62434, respectively; GenBank accession no. AC002454). Restriction mapping allowed us to locate the breakpoint in patients 1 and 3 inside the breakpoint cluster region previously described within a 3.6 kbp upstream of the transcriptional start site of CDK6.[2] As no mitosis was available for FISH analysis in LAN, Igκ light chain locus involvement was confirmed by Southern blot analysis showing the cohybridization of the *CDK6* rearranged band with the Igκ probe (Jκ).

SB indicates Southern blot; R, rearranged; and NA, not available.