Amino Acids (2008) 35: 291–293
DOI 10.1007/s00726-007-0593-1
Printed in The Netherlands

Amino Acids

Human amniotic fluid stem cells: a new perspective

Minireview Article

N. Siegel, M. Rosner, M. Hanneder, A. Freilinger, and **M. Hengstschläger**

Medical Genetics, Obstetrics and Gynecology, Medical University of Vienna, Vienna, Austria

Received July 7, 2007
Accepted July 20, 2007
Published online August 21, 2007; © Springer-Verlag 2007

Summary. The discovery of amniotic fluid stem cells initiated a new and very promising field in stem cell research. In the last four years amniotic fluid stem cells have been shown to express markers specific to pluripotent stem cells, such as Oct-4. Due to their high proliferation potential, amniotic fluid stem cell lineages can be established. Meanwhile, they have been shown to harbor the potential to differentiate into cells of all three embryonic germ layers. It will be a major aim for the future to define the potential of this new source of stem cells for therapies related to specific diseases.

Keywords: Amniotic fluid stem cells – Oct-4 – Differentiation

*

Despite the wide and well-established usage of amniotic fluid cells in routine genetic prenatal diagnosis, almost nothing was known about their origins and properties (Prusa and Hengstschläger, 2002). It was a report from Dario Fauza's group, in 2001, which has initiated a new interest in amniotic fluid cells. The authors seeded a scaffold with amniotic fluid cells derived from pregnant ewes. Cells with an immunocytochemical profile of mesenchymal, fibroblast/myofibroblast cell lineages were isolated and expanded in vitro (Kaviani et al., 2001). When babies are born with body-wall defects, they are often too small for a graft to be taken from elsewhere on their bodies for correctional surgery. These new findings suggested that amniotic fluid cells could become a promising source for autologous fetal tissue engineering.

The first evidence that human amniotic fluid could contain stem cells was provided in 2003. The transcription factor Oct-4 is a marker for pluripotent human stem cells known to be expressed in embryonic carcinoma cells, embryonic germ cells, and embryonic stem cells (Pesce and Schöler, 2001). RT-PCR, Western blot, and immunocytochemical analyses demonstrated that in the background of Oct-4 negative cells, a distinct population of cells expressing Oct-4 can be found in human amniotic fluid. That these cells harbor proliferation potential was suggested by the observation of high levels of co-expressed cyclin A, a well-known proliferation marker (Prusa et al., 2003). In the same year, in t'Anker and colleagues demonstrated that human amniotic fluid contains a cell population positive for mesenchymal markers such as CD90, CD105, CD73, or CD166 but negative for the hematopoietic markers such as CD45, CD34, and CD14. The authors suggested that these mesenchymal stem cells (proven to harbor adipogenic and osteogenic differentiation capacity) could be used for co-transplantation, in conjunction with human umbilical cord blood-derived hematopoietic stem cells (in t'Anker et al., 2003). The existence of a human amniotic fluid stem cell type expressing both, Oct-4 and the markers CD44 and CD105 (but being negative, e.g., for CD34), was first suggested in 2004. These cells could also be induced to differentiate into adipocytes and osteocytes (Tsai et al., 2004). Meanwhile, the presence of Oct-4 positive cells in human amniotic fluid has been confirmed (Karlmark et al., 2005; Bossolasco et al., 2006). Despite these overlapping patterns of marker expressions (Table 1), it remains unproven whether all these studies describe the same cell type. Even more importantly, to really prove that human amniotic fluid contains pluripotent stem cells, it is essential to show that, starting with a single cell, differentiation into different cell lineages can be obtained.

Table 1. Comparison of markers detected in the described human amniotic fluid stem cells

Oct-4	CD34	CD44	CD45	CD105	HLA-ABC	Ref.
+						Prusa et al. (2003)
	−	+	−	+	+	in t'Anker et al. (2003)
+	−	+		+	+	Tsai et al. (2004)
+	−	+	−	+	+	De Coppi et al. (2007)

The recent publication from Anthony Atala's group represents a real breakthrough in amniotic fluid stem cell research (De Coppi et al., 2007). Clonal cell lines that are positive for mesenchymal markers and, again, negative for hematopoietic markers have been isolated. Over 90% of these cells also express the transcription factor Oct-4. This marker pattern again strongly suggests that all these reports describe a very similar amniotic fluid stem cell type (Table 1). Most importantly, using the technique of marking with a retroviral vector, the authors demonstrate for the first time that, descending from a single cell, differentiation along six distinct lineages (adipogenic, osteogenic, myogenic, endothelial, neurogenic, and hepatic) can be induced. This differentiation potential into cells of all three embryonic germ layers together with the observed high proliferation rate are two clear advantages over most of the known adult stem cell sources. Both, embryonic stem cells and these amniotic fluid stem cells, express Oct-4. Importantly, unlike embryonic stem cells, the latter do not form tumors in severe combined immunodeficient mice. In addition, embryonic stem cell research raises profound ethical issues, regarding when human life begins and the moral status of few-days-old embryos.

Taken together, all these data provide evidence that amniotic fluid represents a new and very promising source for stem cell research. Amniotic fluid stem cells are, obviously, an intermediate stage between embryonic stem cells and lineage-restricted adult progenitor cells. However, although all the above-discussed reports presented work on very similar amniotic fluid stem cell populations, evidence for other stem cells in human amniotic fluid already exists: unlike the cells discussed above, which are CD133 negative (De Coppi et al., 2007), another cell type has been detected in human amniotic fluid expressing markers for neuronal stem and progenitor cells, such as CD133, and harboring the potential to differentiate into neurogenic cells (Prusa et al., 2004).

It is important to note that, before speculating about amniotic fluid stem cell banking, further basic research is essential and clinical studies must be initiated. Keeping in mind the well-known risk in amniocentesis to cause a woman to miscarry, it will be most important for the future to invent medical approaches and instruments for collecting amniotic fluid during natural labor of a term patient. However, the data obtained so far clearly warrant projects to investigate the therapeutic potential and usage of these stem cells for specific human diseases. Very recently, the European Union has funded KIDSTEM, a Marie Curie Research Network, including several stem cell research groups from partner institutions from Italy, UK, Germany, and Austria (http://www.kidstem.org). The aim of this project is to design a stem cell based therapy that will prevent end-stage renal failure caused by reflux nephropathy in children. In this leading cause of end-stage renal disease, progression to end-stage renal failure typically takes several years, allowing time for therapies to repair damaged kidneys before they become completely non-functional. The properties of adult kidney stem cells, human embryonic stem cells, and human amniotic fluid stem cells will be investigated to determine which is most appropriate for the generation of functional renal tissue, promoted by specifically designed biomaterials. Recent, fascinating advances make it tempting to speculate that the discussed advantages of amniotic fluid stem cells over embryonic stem cells (tumor development and ethics) and adult stem cells (low proliferation rate and lineage-restricted potential) might probably lead cells from human amniotic fluid to win the race.

Acknowledgements

The current work of the group on human amniotic fluid stem cells is supported by the Research Training Network "Developing a stem cell based therapy to replace nephrons lost through reflux nephropathy" (http://www.kidstem.org) funded by the European Community as part of the Framework program 6 (FP6 036097-2).

References

Bossolasco P, Montemurro T, Cova L, Zangrossi S, Calzarossa C, Miatiotis S, Soligo D, Bosari S, Silani V, Deliliers GL, Rebulla P, Lazzari L (2006) Molecular and phenotypic characterization of human amniotic fluid cells and their differentiation potential. Cell Res 16: 329–336

De Coppi P, Bartsch G, Siddiqui MM, Xu T, Santos TX, Perin L, Mostoslavsky G, Serre AC, Snyder EY, Yoo JJ, Furth ME, Soker S, Atala A (2007) Isolation of amniotic stem cell lines with potential for therapy. Nat Biotechnol 25: 100–106

in t'Anker PS, Scherjon SA, Kleijburg-van der Keur C, Noort WA, Claas FHJ, Willemze R, Fibbe WE, Kanhai HHH (2003) Amniotic fluid as a

novel source of mesenchymal stem cells for therapeutic transplantation. Blood 102: 1548–1549

Karlmark KR, Freilinger A, Marton E, Rosner M, Lubec G, Hengstschläger M (2005) Activation of Oct-4 and Rex-1 promoters in human amniotic fluid cells. Int J Mol Med 16: 987–992

Kaviani A, Perry TE, Dzakovic A, Jennings RW, Ziegler MM, Fauza DO (2001) The amniotic fluid as a source of cells for fetal tissue engineering. J Pediatr Surg 36: 1662–1665

Pesce M, Schöler HR (2001) Oct-4: gatekeeper in the beginning of mammalian development. Stem Cells 19: 271–278

Prusa A, Hengstschläger M (2002) Amniotic fluid cells and human stem cell research – a new connection. Med Sci Monit 8: 253–257

Prusa AR, Marton E, Rosner M, Bernaschek G, Hengstschläger M (2003) Oct-4 expressing cells in human amniotic fluid: a new source for stem cell research? Hum Reprod 18: 1489–1493

Prusa AR, Marton E, Rosner M, Bettelheim D, Lubec G, Pollak A, Bernaschek G, Hengstschläger M (2004) Neurogenic cells in human amniotic fluid. Am J Obstet Gynecol 191: 309–314

Tsai M-S, Lee J-L, Chang Y-J, Hwang S-M (2004) Isolation of human multipotent mesenchymal stem cells from second-trimester amniotic fluid using a novel two-stage culture protocol. Hum Reprod 19: 1450–1456

Authors' address: Prof. M. Hengstschläger, Medical Genetics, Obstetrics and Gynecology, Medical University of Vienna, Währinger Gürtel 18–20, 1090 Vienna, Austria,

Fax: +43-1-40400-7848, E-mail: markus.hengstschlaeger@meduniwien.ac.at