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## Stem Cells in Amniotic Fluid - What are the Next Steps to Do?

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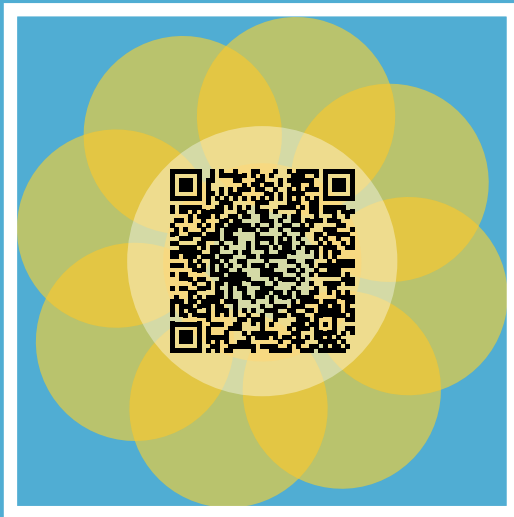
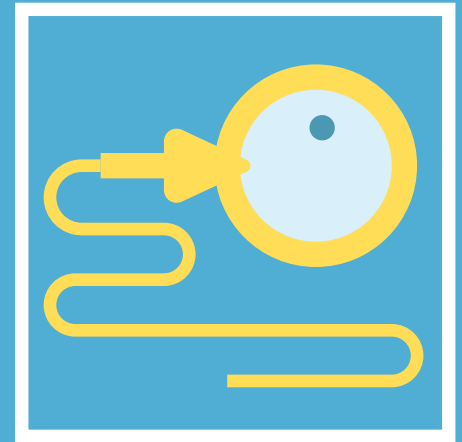
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## BACK TO THE FUTURE

# Stem Cells in Amniotic Fluid – What are the Next Steps to Do?

M. Hengstschläger

*It is the hope of patients and investigators that in future the characterisation and isolation of human stem cells will allow the establishment of new therapeutic concepts for a wide variety of diseases. Recently, we found a new source for stem cells. Human amniotic fluid contains cells, which express Oct-4, a marker for pluripotent stem cells. In addition, we described amniotic fluid cells expressing markers for neuronal stem cells. The latter harbour the potential to differentiate into neurogenic cells. This opened a new field in stem cell research. In this review I want to summarise the current knowledge about amniotic fluid cells focusing on the open questions, which need to be investigated in future. J Reproduktionsmed Endokrinol 2005; 2 (4): 233–8.*

**Key words:** stem cells, amniotic fluid, Oct-4, differentiation potential

It is easy to imagine that undifferentiated and differentiated cells of varying lineages are present in human amniotic fluid. Although for the mixture of these cells a wide variety of different origins has been suggested, for many subsets of amniotic fluid cells the embryonic/fetal origins remain unclear. Even in pregnancies with normal fetal development, the cells in the amniotic fluid are heterogeneous. Evidence has been provided that ectodermal, mesodermal as well as endodermal cells can be found in amniotic fluid depending e. g. on the gestational age or on fetal pathologies. Cells belonging to e. g. the amnion, skin, the urogenital, respiratory, and digestive systems have been reported to be detectable. It has been found that in cases of certain fetal abnormalities, including an open lesion, such as a neural tube defect, or an abdominal wall abnormality, such as gastroschisis, the morphology and growth properties of the cells in the amniotic fluid are different [1, 2].

Amniotic fluid cells are used for prenatal diagnosis of a wide range of fetal abnormalities caused by genetic mutations. In the course of cultivation of human amniotic fluid cells for routine prenatal genetic diagnosis, adhering and dividing colony-forming cells are selected. Accordingly, in the past scientific interest was focused on the characterisation of these cells. Still, despite the wide and well established usage of human amniotic fluid cells in routine prenatal diagnosis, the current knowledge about origin and properties of these cells is limited. Early classifications of these cells were mainly based on morphological criteria, and are thus inadequate. Only very limited biochemical data on these cells are available. A mixture of morphological aspects, limited biochemical criteria, and growth characteristics led to the classification of amniotic fluid cells, which attach and form colonies under routine culture conditions, into epitheloid cells, amniotic fluid specific cells, and fibroblastic cells. Amniotic fluid specific cells and epitheloid cells appear at the beginning of cultivation, but only the amniotic fluid specific cells persist during extended cultivation process. Fibroblastic cells cannot be cultivated from every amniocentesis sample. Although it is believed that there are as yet unknown additional origins for all three cell types, epitheloid cells have been thought to derive from fetal skin and urine. Amniotic fluid specific cells have been considered to derive from fetal membranes and

trophoblasts, and fibroblastic cells from fibrous connective tissue and dermal fibroblasts. The hypothesis that amniotic fluid specific cells originate from placental trophoblast tissue has been supported by the finding that they produce estrogen, human chorionic gonadotropin and progesterone. Also due to the lack of hormone production, fibroblastic cells are considered to originate from mesenchymal tissue, while both types express HLA Class I (HLA-ABC) surface antigens, but not HLA Class II (HLA-DR) [1–6].

Unfortunately, until recently almost nothing was known about those cells within native amniotic fluid, which are incapable of attachment and proliferation under the cell culture conditions used in routine prenatal laboratory diagnosis. Here it is very important to note, that only a very small amount of all the cells present in human amniotic fluid form colonies under routinely used cell culture conditions. In addition, it is possible that specific cells in the amniotic fluid have the capacity to attach, but do not proliferate or form colonies because of cell cycle arrest, differentiation status, or senescence. Many of these cells are viable, as can be concluded from trypan blue exclusion assays [1, 2, 4].

Our first approach to get more information on the spectrum of proteins/cells present in human amniotic fluid made use of the proteomic technology. Human amniotic fluid cells obtained from amniocentesis for routine prenatal genetic diagnosis were subjected to proteomic analysis. This project has been reviewed and accepted by the ethics commission of the Medical University of Vienna, Austria. To identify metabolic enzymes, two-dimensional gel electrophoresis with in-gel digestion followed by matrix-assisted laser desorption/ionisation-time of flight analysis was performed. The 99 unambiguously identified proteins were grouped into different categories, such as carbohydrate handling enzymes, amino acid handling enzymes, proteins of purine metabolism, enzymes of intermediary metabolism and miscellaneous. Since numerous metabolic derangements are known to be associated with Down Syndrome, human amniotic fluid cells with trisomy 21 have also been included into this study. In trisomic amniotic fluid cells ribose-phosphate pyrophosphokinase I, phosphoserine aminotransferase, lactate dehydrogenase, pyruvate kinase M1 isoenzyme, and glyceraldehyde 3-phosphate dehydrogenase were signi-

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ificantly increased. Downregulation in cells with an additional chromosome 21 was observed for glutamate dehydrogenase, UDP-glucose 6 dehydrogenase, ATP synthase  $\alpha$  chain, phosphoribosylformylglycinamide synthase, and ATP synthases  $\alpha$  and  $\beta$  chain [7]. This characterisation not only provides new insights into the spectrum of proteins included in human amniotic fluid cell samples, it also provides an important basis for further investigations with the aim to characterise and isolate specific subsets of human amniotic fluid cells. Such an approach, with the advantage of the concomitant determination of many enzyme proteins, may also form the basis for future metabolic screens when amniocentesis is carried out. Down Syndrome is the most frequent genetic cause of mental retardation and a demonstration of deregulated metabolic enzymes could also provide the basis for further investigations of the molecular development of this disease.

### First Evidence for Stem Cells in Amniotic Fluid

One cell type that is also believed to be part of the cellular content of amniotic fluid is the human amniotic epithelial cell. These cells are formed from amnioblasts, separated from the epiblast at about the 8<sup>th</sup> day after fertilisation and constitute the inner layer of the amnion [1–6]. Why these cells can be detected in human amniotic fluid remains elusive. Human amniotic epithelial cells have been speculated to have stem cell potential because they can differentiate into a wide variety of different organs, including heart, liver and brain [8]. Amniotic epithelial cells have been demonstrated to express neuronal stem cell markers and synthesise and release acetylcholine and catecholamines [9–11]. The two latter have also been detected in amniotic fluid and in amniotic fluid cells [12]. In addition, neurotrophic factors, such as nerve growth factor, brain derived neurotrophic factor, and neurotrophin-3, have been detected in both, human amniotic epithelial cells and human amniotic fluid [13, 14]. Is this overlap in biochemical characteristics due to amniotic epithelial cells in amniotic fluid or caused by two different cell types? Do cells in human amniotic fluid also express neuronal stem cell markers? Especially the latter question initiated further investigations in our laboratory (see below). This is of special interest, because human amniotic epithelial cells have already been shown to be able to survive and function in the brain of a rat model of Parkinson's disease [15, 16].

Telomerase is an enzyme that helps maintaining telomeres, which protect the ends of chromosomes. Telomerase activity is an important marker for human pluripotent stem cells and is – together with long telomeres – characteristic of proliferating cells in embryonic tissues and of germ cells. Human non-transformed somatic cells, however, do not show telomerase activity and their telomeres are considerable shorter [17–19]. Another important evidence that human amniotic fluid could contain stem cells was provided by the detection of telomerase activity in both uncultured and cultured human amniotic fluid cells from 14 weeks' gestation [20]. To confirm and more detailly investigate this issue, we performed RT-PCR analyses with human amniotic fluid cell samples and cells from human placental biopsies from pregnancies from 13<sup>th</sup> up to the 34<sup>th</sup> week of gestational age. We demonstrated that these samples contain cells expressing human telomerase reverse transcriptase, bone morpho-

genetic protein receptor II (known to be important for the differentiation of committed mesenchymal cell types from mesenchymal stem and progenitor cells) and the leukaemia inhibitory factor (a growth factor necessary for maintaining embryonic stem cells in a proliferative, undifferentiated state). Deregulation of specific differentiation processes is a major cause for the neuropathological cell features typical for Down Syndrome, the most frequent genetic cause of mental retardation. The molecular mechanisms leading to Down Syndrome are likely to be operative from the very earliest time of embryonic/fetal development. However, we could not detect any significant differences in the expression of the stem cell markers described above between normal and trisomy 21 cell samples. These data suggest that early deregulation of the expression of these genes in the here analysed cell sources does not contribute to the molecular development of Down Syndrome [21].

A very interesting study demonstrated that amniotic fluid is able to serve as a source for cells for fetal tissue engineering. Amniotic fluid cells from pregnant ewes were grown in culture, and a specific subpopulation of morphologically distinct cells was mechanically isolated and selectively expanded under specific culture conditions. Here it is important to note, that this procedure leads to the enrichment of a very specific subpopulation of cells out of the wide spectrum of cells, which can be found in amniotic fluid. The determined immunocytochemical profile of the cells enriched in this way was consistent with a mesenchymal, fibroblast/myofibroblast cell lineage. These cells stained positive for vimentin, smooth muscle actin, cytokeratin 8 and 18, and fibroblast surface protein, and negatively for desmin and cluster differentiation 31. They exhibited significantly faster proliferation than comparable fetal and adult cells. After expansion, these cells were seeded onto a polyglycolic acid polymer/poly-4-hydroxybutyrate scaffold. Scanning electron microscopy revealed dense, confluent layers of cells surrounding the polymer matrices and firm cell adhesion of both scaffolds [22]. Such a biological construct could have applications in a variety of congenital anomalies. In case of prenatal ultrasound diagnosis of e. g. congenital diaphragmatic hernia or body wall defects, amniotic fluid could serve as a source to create an engineered construct – material containing cells from the fetus, to function as a graft – for implantation immediately after birth [23].

### Cells within the Human Amniotic Fluid Express Oct-4

The Oct-4 POU transcription factor is a marker for pluripotent human stem cells. Embryonal carcinoma cells, embryonic germ cells and embryonic stem cells are known to express Oct-4. Oct-4 expression rapidly disappears when stem cells start to differentiate [24, 25]. In a recently published study of our group [26] we described that in human amniotic fluid in the background of Oct-4 negative cells a distinct population of cells can be found, which express Oct-4. We detected Oct-4 mRNA expression in amniotic fluid cell samples via RT-PCR. Western blot analyses demonstrated Oct-4 protein expression in samples with positive RT-PCR results. Immunocytochemical analyses of positive samples revealed that the Oct-4 signals were localised to the nucleus, what is in agreement with the known localisation of this POU transcription factor [25] (Fig. 1). Double staining experiments

further demonstrated that the found Oct-4 positive amniotic fluid cells actively divide. We showed that the Oct-4 positive cells also express cyclin A, a marker for cell cycle proliferation [27]. Using RT-PCR analyses we further demonstrated that in amniotic fluid cell samples containing Oct-4 positive cells, the mRNAs of the following three genes can be detected [26]: stem cell factor known to be expressed in embryonic stem cells, embryonic carcinoma cells, haematopoietic stem cells and mesenchymal stem cells [28], vimentin known to be expressed in ectodermal, neural and pancreatic progenitor cells [29] and alkaline phosphatase known to be expressed in embryonic stem cells and in embryonic carcinoma cells [17].

Our finding that human amniotic fluid contains Oct-4 positive cells has been confirmed by a study of another research group [30]. The authors established a two-stage culture protocol to isolate human multipotent mesenchymal stem cells from second-trimester amniotic fluid. Mesenchymal stem cells are multipotent stem cells, able to differentiate into multiple mesenchymal lineages. Oct-4 mRNA expression was detected in all 20 analysed independent cases. Immunocytochemical analyses further demonstrated Oct-4 protein expression in these cells.

These results suggest that human amniotic fluid may represent a new source for the isolation of human Oct-4 positive stem cells without raising the ethical concerns associated with human embryonic research. Considering the great potential of cellular therapy using fetal stem cells and the feasibility of intrauterine fetal tissue engineering, amniotic fluid may provide an excellent alternative source for investigation of human stem cells. However, several open questions still must be investigated. Final proof for a putative pluripotential of such Oct-4 positive amniotic fluid cells would include experiments to isolate them and to differentiate them into distinct lineages (see below). Although probably associated with a lot of experimental difficulties, approaches should also aim to identify the biological origin of such cells in the human amniotic fluid.

### **Mesenchymal Stem Cells in Human Amniotic Fluid**

As described above, multipotent mesenchymal stem cells were successfully isolated, cultured and enriched from human amniotic fluid. Flow cytometry analyses showed that they were positive for SH2, SH3, SH4, CD29, CD44, HLA-ABC (MHC class I), CD90 and CD105. These cells could be induced to differentiate into adipocytes, osteocytes and neuronal cells [30].

Additional evidence for mesenchymal stem cells in human amniotic fluid was provided by another recent study. It was shown that a quantity of 2 ml of second-trimester human amniotic fluid was sufficient to culture mesenchymal stem cells. This expansion potential even exceeded that of bone marrow-derived mesenchymal stem cells. Phenotypical characterisation by flow cytometry revealed that these amniotic fluid-derived stem cells are very similar to mesenchymal stem cells derived from second-trimester fetal tissues and adult bone marrow. Further cultivation in different specific cell culture media demonstrated multilineage differentiation potential of amniotic fluid-derived mesenchymal stem cells

into fibroblasts, adipocytes and osteocytes [31]. The frequency of mesenchymal stem cells in umbilical cord blood is particularly low, and most investigators have been unable to grow mesenchymal stem cells from umbilical cord blood. Fetal lung-derived mesenchymal stem cells can enhance the engraftment of human umbilical cord blood-derived CD34<sup>+</sup>-haematopoietic cells in severe combined immunodeficiency mice [32]. Accordingly, cotransplantation of umbilical cord blood and mesenchymal stem cells derived from parental bone marrow is proposed as a strategy to reduce the delay in engraftment that is associated with umbilical cord blood transplantation. The demonstration of mesenchymal stem cells in second-trimester amniotic fluid could enable cotransplantation of haematopoietic stem cells from umbilical cord blood and mesenchymal stem cells from amniotic fluid from the same donor.

### **Amniotic Fluid as a New Source for Neural Stem Cells**

We recently performed a study to determine whether human amniotic fluid contains cells that harbor the potential to differentiate into neurogenic cells. Amniotic fluid cells, uncultivated or cultivated either in medium standard for routine diagnosis or in neurogenic differentiation medium, were analysed for morphologic neurogenic differentiation and for the expression of specific markers. Whereas the appearance of neurogenic cells was not detected in uncultivated cells, it strongly increased after incubation with neurogenic medium. RT-PCR and Western blot analyses of the expression of CD133 (a marker for neuronal stem cells), nestin (characteristic for neuronal progenitor cells), neurofilament (found in differentiated neurons), CNPase (characteristic for oligodendrocytes), and the p75 common neurotrophin receptor, the brain-derived neurotrophic factor and neurotrophin-3, which are known to be important for the differentiation and survival of neurons, revealed induction of these markers upon treatment with neurogenic differentiation medium. Accordingly, the marker gene expression was connected with the appearance of the characteristic morphologic changes [33]. For the first time, our study demonstrated that human amniotic fluid contains cells that express markers for neuronal stem and progenitor cells, which harbour the potential to differentiate into neurogenic cells.

### **The Next Questions to Answer**

Much of the recent excitement surrounding human stem cells is connected with the hope of investigators and patients alike that they have the potential to treat or cure a myriad of diseases. The fact that the probably most potent human stem cells can be derived from the inner cell mass during embryonic development, raises a lot of ethical issues. Accordingly, investigations have been initiated to search for different human sources for cells harbouring the potency to differentiate into specific lineages [34, 35]. One such alternative source could be human amniotic fluid. The observations described above suggest that human amniotic fluid may represent a new source for the isolation of cells with the potency to differentiate into distinct cells. Such cells could be used as primary (not transformed, not immortalised) cell systems for basic research and they could probably provide a new source for stem

cell research and for the development of new approaches in tissue engineering.

For the future it is of great importance to obtain more insights into the spectrum of cells contained in human amniotic fluid. Using RT-PCR the expression of a wide variety of genes, known as markers for stem cells, progenitor cells and differentiated cell types, should be analysed. In this respect a general issue of relevance is that fetal anomalies, such as e. g. open lesions including neural tube defects or the abdominal wall abnormality gastroschisis, very likely have significant impact on the spectrum of cells in the fluid. This aspect should be investigated by including amniotic fluid cell samples of such anomalies into a RT-PCR screen. Human amniotic fluid cells can be obtained from amniocentesis all performed at different time points after the 14<sup>th</sup> week of pregnancy for routine prenatal diagnosis. Using such an approach it has already been published that human amniotic fluid cell samples can be positive for the expression of Oct-4, alkaline phosphatase, stem cell factor, vimentin, CD133, telomerase reverse transcriptase, bone morphogenetic protein receptor II, the leukaemia inhibitory factor, nestin, neurofilament, CNPase, and the p75 common neurotrophin receptor, the brain-derived neurotrophic factor and neurotrophin-3 [21, 26, 33]. It is important that different research groups start to perform a wide screen to determine which markers are expressed in human amniotic fluid cells. In Table 1 I suggest a list of markers, which should be included in such studies.

Furthermore, it is important to investigate whether the level of the expression of the above mentioned markers changes during pregnancy (comparison of earlier and later amniocentesis material) or is affected by specific fetal abnormalities (such as e. g. neural tube defects or gastroschisis). An optimal approach to answer these questions would be quantitative RT-PCR [37]. A next step would be to clarify whether observed differences are due to different gene expression levels or to different concentrations of marker-positive cells in the amniotic fluid. This issue must be investigated by immunohistochemistry [26].

**Table 1.** List of markers (compare e. g. [17, 26, 29, 36])

**Pluripotent stem cells:**

Oct-4, alkaline phosphatase, stem cell factor, vimentin, telomerase, Rex-1

**Neural stem cells/haematopoietic stem cells:**

CD133

**Neuronal progenitor cells:**

Nestin

**Neurons:**

Neurofilament

**Oligodendrocytes:**

Cyclic nucleotide phospho-diesterase (CNPase)

**Astrocytes:**

Glial fibrillary acidic protein (GFAP)

**Vascular:**

CD34

**Osteogenesis:**

Osteocalcin, osteonectin, core-binding-protein  $\alpha$ 1

**Chondrogenesis:**

Collagen type2, aggrecan, decorin, biglycan

**Muscle cells:**

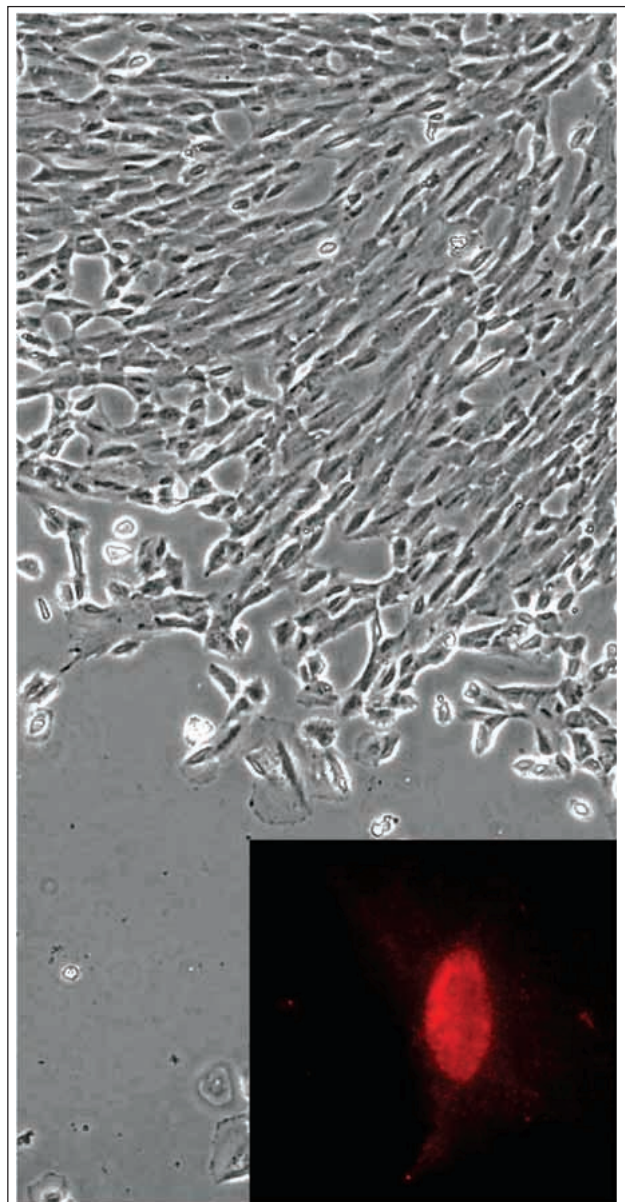
Myogenin, MyoD, myosin heavy chain

**Adipocytes:**

Peroxisome-proliferating activated receptor  $\gamma$ 1 and  $\gamma$ 2, lipoprotein lipase, leptin

The entire experiments described above should be performed on mRNA extracts of cells of the native amniotic fluid sample and of cells of the (same) sample after cultivation for routine prenatal genetic testing. This analysis should provide new detailed insights into which cells could be part of the spectrum found in human amniotic fluid. This detailed screen should provide a basis for further research on characterisation and isolation of specific subsets of cells expressing specific markers.

Screening techniques such as microarray approaches to determine mRNA expression differences or proteomics to detect regulation in protein levels and modifications should also be performed with different samples of amniotic fluid cells (normal versus pathological, earlier versus later pregnancy etc.) [7, 37].



**Figure 1.** Detection of Oct-4 in the nucleus of a human amniotic fluid cell. A phase contrast picture of an amniotic fluid cell population grown under culture conditions used for routine prenatal diagnosis of genetic mutations is presented. For immunocytochemical analysis of cellular Oct-4 expression, cells were fixed and incubated with anti-Oct-4 antibody. Thereafter, cells were washed, incubated with a biotinylated secondary antibody, washed again, and incubated with ExtrAvidin-Cy3 conjugate. One amniotic fluid cell with the red Oct-4-specific signal in the nucleus is shown.

Our data, whereupon human amniotic fluid contains Oct-4 positive cells, have already been confirmed by others [26, 30]. A major issue for the future is to detailly investigate the differentiation potential of the found Oct-4 positive amniotic fluid cells. Therefore, it is essential to think about strategies to isolate/enrich Oct-4 positive cells out of amniotic fluid. One could think about transfecting amniotic fluid cell samples with a plasmid harbouring a neomycin resistance gene under the control of the Oct-4 promoter. Cultivation in a medium containing neomycin enables selection for Oct-4 positive cells. The successful approach to use this construct to isolate Oct-4 positive stem cells out of a background of Oct-4 negative cells is e. g. described in [38]. Further cultivation of amniotic Oct-4 positive cells is possible because these cells divide as shown by co-staining with cyclin A [26]. Transfection of a plasmid harbouring GFP under the control of the Oct-4 promoter could enable the sorting of GFP positive (Oct-4 positive) cells via flow activated cell sorting. The successful usage of this approach to isolate Oct-4 positive stem cells out of a majority of Oct-4 negative cells has already been described earlier [39]. The Rex-1 gene is a transcriptional target of the transcription factor Oct-4 and is expressed in human embryonic stem cells [40–42]. Accordingly, in the past a construct harbouring GFP under the control of the Rex-1 gene promoter has successfully been used to isolate human multipotent stem cells [43]. This construct could also be tested in human amniotic fluid cell samples.

As a logical consequence it will be very important to define the differentiation potential of these (so isolated) cells. Cells, selected for Oct-4 expression or sorted according to Oct-4 or Rex-1 expression, must then be transferred in distinct differentiation media, such as those described in Table 2. RT-PCR analyses of the markers described in Table 1 will allow to investigate whether these cells harbour the potential for neurogenic, osteogenic, chondrogenic, myogenic and/or adipogenic differentiation.

In addition, starting from one cell (obtained via cell sorting or minimal dilution after sorting) different cell cultivations in the specific differentiation media (Tab. 2) should be performed after cell culture splitting. Again, marker gene expression can be performed to confirm cellular differentiation. If started from one cell, a cell culture can differentiate in two or more different lineages this would be a proof for the existence of stem/progenitor cells with multi-lineage capacity in human amniotic fluid. Recently, comparable experiments demonstrated that human adipose tissue is a source of multipotent stem cells [36]. These data showed that fat tissue extracted during liposuction is a rich source of what has been char-

acterised as adipose-derived stem cells. Experiments, as those described above, demonstrated that these cells can differentiate into several lineages. Since then a handful of laboratories and biotechnology companies are working to exploit the potential of these stem cells [46]. Very comparable to adipose-derived stem cells, amniotic fluid-derived stem cells still need a lot of science and medicine to generate more data on so far unresolved issues.

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## References:

- Gosden CM. Amniotic fluid cell types and culture. *Br Med Bull* 1983; 39: 348–54.
- Prusa AR, Hengstschläger M. Amniotic fluid cells and human stem cell research: a new connection. *Med Sci Monit* 2002; 8: RA 253–7.
- Milunsky A. Amniotic fluid cell culture. In: Milunsky A (ed). *Genetic disorder and the fetus*. Plenum Press, New York, 1979.
- Hoehn H, Salk D. Morphological and biochemical heterogeneity of amniotic fluid cells in culture. *Methods Cell Biol* 1982; 26: 11–34.
- Thakar N, Priest RE, Priest JH. Estrogen production by cultured amniotic fluid cells. *Clin Res* 1982; 30: 888A.
- Whitsett CF, Priest RE, Priest JH, Marion J. HLA-typing of cultured amniotic fluid cells. *Am J Clin Pathol* 1983; 79: 186.
- Oh JE, Fountoulakis M, Juranville JF, Rosner M, Hengstschläger M, Lubec G. Proteomic determination of metabolic enzymes of the amnion cell: basis for a possible diagnostic tool? *Proteomics* 2004; 4: 1145–8.
- Sandler TW. *Langman's Medical Embryology*. William and Wilkins, Baltimore, MD, 1995; 71–80.
- Sakuragawa N, Thangavel R, Mizuguchi M, Hirasawa M, Kamo I. Expression of markers for both neuronal and glial cells in human amniotic epithelial cells. *Neurosci Lett* 1996; 209: 9–12.
- Sakuragawa N, Misawa H, Ohsugi K, Kakishita K, Ishii T, Thangavel R, Tohyama J, Elwan M, Yokoyama Y, Okuda O, Arai H, Ogino I, Sato K. Evidence for active acetylcholine metabolism in human amniotic epithelial cells: applicable to intracerebral allografting for neurologic disease. *Neurosci Lett* 1997; 232: 53–6.
- Elwan MA, Sakuragawa N. Evidence for synthesis and release of catecholamines by human amniotic epithelial cells. *Neuroreport* 1997; 8: 3435–8.
- Sakuragawa N, Elwan MA, Fujii T, Kawashima K. Possible dynamic neurotransmitter metabolism surrounding the fetus. *J Child Neurology* 1999; 11: 265–66.
- Uchida S, Inanaga Y, Kobayashi M, Hurukawa S, Araie M, Sakuragawa N. Neurotrophic function of conditioned medium from human amniotic epithelial cells. *J Neurosci Res* 2000; 62: 585–90.
- Marx CE, Vance BJ, Jarskog LF, Chescheir NC, Gilmore JH. Nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3 levels in human amniotic fluid. *Am J Obstet Gynecol* 1999; 181: 1225–30.
- Kakishita K, Elwan M, Nakao N, Itakura T, Sakuragawa N. Human amniotic epithelial cells produce dopamine and survive after implantation into the striatum of a rat model of Parkinson's disease: a potential source of donor for transplantation therapy. *Exp Neurol* 2000; 165: 27–34.
- Kakishita K, Nakao N, Sakuragawa N, Itakura T. Implantation of human amniotic epithelial cells prevents the degeneration of nigral dopamine neurons in rats with 6-hydroxydopamine lesions. *Brain Res* 2003; 980: 48–56.
- Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; 284: 143–7.
- Monk M, Holding C. Human embryonic genes re-expressed in cancer cells. *Oncogene* 2001; 20: 8085–91.
- Amit M, Carpenter MK, Inokuma MS, Chiu CP, Wanknitz MA, Itskovitz-Eldor J, Thompson JA. Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. *Dev Biol* 2000; 227: 271–8.

**Table 2.** Media for induction of lineage-specific differentiation (compare e. g. [33, 36, 44, 45])

<b>Neurogenic differentiation:</b>	– medium with low serum containing dimethylsulfoxid
<b>Osteogenic differentiation:</b>	– medium containing 1,25-dihydroxyvitamin D3, ascorbate-2-phosphate, $\beta$ -glycerophosphate
<b>Chondrogenic differentiation:</b>	– medium with low serum containing insulin, TGF $\beta$ 1, ascorbate-2-phosphate
<b>Myogenic differentiation:</b>	– medium containing hydrocortisone
<b>Adipogenic differentiation:</b>	– medium containing isobutyl-methylxanthine, dexamethasone, insulin, indomethacin

20. Mosquera A, Fernandez JL, Campos A, Goyanes VJ, Ramiro-Diaz J, Gosalvez J. Simultaneous decrease of telomere length and telomerase activity with ageing of human amniotic fluid cells. *J Med Genet* 1999; 36: 494–6.
21. Prusa AR, Marton E, Rosner M, Freilinger A, Bernaschek G, Hengstschlager M. Stem cell marker expression in human trisomy 21 amniotic fluid cells and trophoblasts. *J Neural Transm* 2003; 67 (Suppl): 235–42.
22. Kaviani A, Perry TE, Dzakovic A, Jennings RU, Ziegler MM, Fauza DO. The amniotic fluid as a source of cells for fetal tissue engineering. *J Pediatr Surg* 2001; 36: 1662–5.
23. Mitka M. Amniotic cells show promise for fetal tissue engineering. *J Am Med Assoc* 2001; 286: 2083.
24. Donovan PJ. High Oct-ane fuel powers the stem cell. *Nature Genet* 2001; 29: 246–7.
25. Pesce M, Scholer HR. Oct-4: gatekeeper in the beginnings of mammalian development. *Stem Cells* 2001; 19: 271–8.
26. Prusa AR, Marton E, Rosner M, Bernaschek G, Hengstschlager M. Oct-4-expressing cells in human amniotic fluid: a new source for stem cell research? *Hum Reprod* 2003; 18: 1489–93.
27. Hengstschlager M, Braun K, Soucek T, Miloloza A, Hengstschlager-Otttnad E. Cyclin-dependent kinases at the G1-S transition of the mammalian cell cycle. *Mutat Res* 1999; 436: 1–9.
28. Martin FH, Suggs SV, Langley KE, Lu HS, Ting J, Okino KH, Morris CF, McNiece IK, Jacobsen FW, Mendiaz EA et al. Primary structure and functional expression of rat and human stem cell factor DNAs. *Cell* 1990; 63: 203–11.
29. Shambloott MJ, Axelman J, Littlefield JW, Blumenthal PD, Huggins GR, Cui Y, Cheng L, Gearhart JD. Human embryonic germ cell derivatives express a broad range of developmentally distinct markers and proliferate extensively in vitro. *Proc Natl Acad Sci USA* 2001; 98: 113–8.
30. Tsai M-S, Lee J-L, Chang Y-J, Hwang S-M. Isolation of human multipotent mesenchymal stem cells from second-trimester amniotic fluid using a novel two-stage culture protocol. *Hum Reprod* 2004; 19: 1450–6.
31. In't Anker PS, Scherjon SA, Kleijburg-Van der Keur C, Noort WA, Claas FHJ, Willemze R, Fibbe WE, Kanhai HHH. Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation. *Blood* 2003; 102: 1548–9.
32. Noort WA, Kruijselbrink AB, In't Anker PS, Kruger M, Van Bezooijen RL, De Paus RA, Heemskerck MH, Lowik CW, Falkenburg JH, Willemze R, Fibbe WE. Mesenchymal stem cells promote engraftment of human umbilical cord blood-derived CD34(+) cells in NOD/SCID mice. *Exp Hematol* 2002; 30: 870–8.
33. Prusa AR, Marton E, Rosner M, Bettelheim D, Lubec G, Pollak A, Bernaschek G, Hengstschlager M. Neurogenic cells in human amniotic fluid. *Am J Obstet Gynecol* 2004; 191: 309–14.
34. Weissman IL. Stem cells: units of development, units of regeneration, and units in evolution. *Cell* 2000; 100: 157–68.
35. Rosenthal N. Prometheus' vulture and the stem-cell promise. *N Engl J Med* 2003; 349: 267–74.
36. Zuk PA, Zhu M, Ashjian P, DeUgarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 2002; 13: 4279–95.
37. Rosner M, Hofer K, Kubista M, Hengstschlager M. Cell size regulation by the human TSC tumor suppressor proteins depends on PI3K and FKBP38. *Oncogene* 2003; 22: 4786–98.
38. Buehr M, Nichols J, Stenhouse F, Mountford P, Greenhalgh CJ, Kantachuvesiri S, Brooker G, Mullins J, Smith AG. Rapid loss of Oct-4 and pluripotency in cultured rodent blastocytes and derivative cell lines. *Biol Reprod* 2003; 68: 222–9.
39. Boiani M, Eckhardt S, Scholer HR, McLaughlin KJ. Oct4 distribution and level in mouse clones: consequences for pluripotency. *Genes Dev* 2002; 16: 1209–19.
40. Hosler BA, Larosa GJ, Grippo JF, Gudas LJ. Expression of Rex-1, a gene containing zinc finger motifs, is rapidly reduced by retinoic acid in F9 teratocarcinoma cells. *Mol Cell Biol* 1989; 9: 5623–9.
41. Ben-Shushan E, Thompson JR, Gudas LJ, Bergman Y. Rex-1, a gene encoding a transcription factor expressed in the early embryo, is regulated via Oct-3/4 and Oct-6 binding to an octamer site and a novel protein, Rox-1, binding to an adjacent site. *Mol Cell Biol* 1998; 18: 1866–78.
42. Du Z-W, Cong H-C, Yao Z. Identification of putative downstream genes of Oct-4 by suppression-subtractive hybridization. *Biochem Biophys Res Com* 2001; 282: 701–6.
43. Eiges R, Schuldiner M, Drukker M, Yanuka O, Itskovitz-Eldor J, Benvenisty N. Establishment of human embryonic stem cell-transfected clones carrying a marker for undifferentiated cells. *Curr Biol* 2001; 11: 514–8.
44. Kubista M, Rosner M, Kubista E, Bernaschek G, Hengstschlager M. Brca1 regulates in vitro differentiation of mammary epithelial cells. *Oncogene* 2002; 21: 4747–56.
45. Soucek T, Holzl G, Bernaschek G, Hengstschlager M. A role of the tuberous sclerosis gene-2 product in neuronal differentiation. *Oncogene* 1998; 16: 2197–204.
46. Ault A. Tapping a stem cell goldmine. Some companies are betting that fat-derived stem cells could have therapeutic payoff. *The Scientist* 2004; 6: 38–9.



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