Amino Acids (2012) 42:1591–1596
DOI 10.1007/s00726-011-0929-8

MINIREVIEW ARTICLE

Neurogenic differentiation of amniotic fluid stem cells

M. Rosner · M. Mikula · A. Preitschopf · M. Feichtinger · K. Schipany · M. Hengstschläger

Received: 13 April 2011 / Accepted: 28 April 2011 / Published online: 15 May 2011

Abstract In 2003, human amniotic fluid has been shown to contain stem cells expressing Oct-4, a marker for pluripotency. This finding initiated a rapidly growing and very promising new stem cell research field. Since then, amniotic fluid stem (AFS) cells have been demonstrated to harbour the potential to differentiate into any of the three germ layers and to form three-dimensional aggregates, so-called embryoid bodies, known as the principal step in the differentiation of pluripotent stem cells. Marker selection and minimal dilution approaches allow the establishment of monoclonal AFS cell lineages with high proliferation potential. AFS cells have a lower risk for tumour development and do not raise the ethical issues of embryonic stem cells. Compared to induced pluripotent stem cells, AFS cells do not need exogenic treatment to induce pluripotency, are chromosomal stable and do not harbour the epigenetic memory and accumulated somatic mutations of specific differentiated source cells. Compared to adult stem cells, AFS can be grown in larger quantities and show higher differentiation potential. Accordingly, in the recent past, AFS became increasingly accepted as an optimal tool for basic research and probably also for specific cell-based therapies. Here, we review the current knowledge on the neurogenic differentiation potential of AFS cells.

Keywords Amniotic fluid · Differentiation · Neurogenic · Neuron · Pluripotent · Stem cell

M. Rosner · M. Mikula · A. Preitschopf · M. Feichtinger · K. Schipany · M. Hengstschläger (✉)
Institute of Medical Genetics, Medical University of Vienna, Währinger Strasse 10, 1090 Vienna, Austria
e-mail: markus.hengstschlaeger@meduniwien.ac.at

Introduction

The first suggestion of human amniotic fluid as a new and potent putative source for human stem cells was published in 2002 (Prusa and Hengstschläger 2002). At this time, before the discovery of induced pluripotent stem (iPS) cells, it was argued that compared to ES cells the differentiation potential and the proliferative capacity of adult stem cells are limited, whereas they do not raise ethical concerns and harbour a lower risk of tumour development. Accordingly, different studies and approaches were initiated with an aim to identify other sources for stem cells with high differentiation potential. These efforts led to the discovery of AFS cells and iPS cells (reviewed in Siegel et al. 2008; Pappa and Anagnou 2009; Dobreva et al. 2010; Mattis and Svendsen 2011).

Amniotic fluid cells were widely used in routine genetic prenatal diagnosis although the knowledge about cell types and their properties was very limited. In 2003, first evidence for stem cells in human amniotic fluid was provided. It was demonstrated that in the background of other cells within human amniotic fluid a distinct highly proliferative stem cell type is viable expressing the pluripotent stem cell marker Oct-4 (Prusa et al. 2003a). It was fascinating to see how fast and progressively growing this new promising stem cell research field was since this first description. On the one hand, several independent publications immediately confirmed the finding of Oct-4-positive stem cells in amniotic fluid (Tsai et al. 2004; Karlmark et al. 2005; Bossolasco et al. 2006; Kim et al. 2007; De Coppi et al. 2007 and many reports thereafter). On the other hand, besides neurogenic differentiation (for a detailed discussion see below), stem cells within amniotic fluid have been demonstrated to harbour the potential to differentiate into osteogenic, chondrogenic, adipogenic, hepatic, myogenic,

renal, and hematopoietic lineages (In 't Anker et al. 2003; Tsai et al. 2004; Bossolasco et al. 2006; Kim et al. 2007; Kolambkar et al. 2007; De Coppi et al. 2007; Perin et al. 2007, 2010; Siegel et al. 2009, 2010; Ditadi et al. 2009; Hauser et al. 2010).

Pluripotency of AFS cells

When native unselected cell mixtures, such as amniotic fluid cell samples, are analysed for their differentiation potential, it is important to keep in mind that the in vitro differentiation procedure could rather reflect a selection of a cell type, which was already part of the original sample, than the development of an undifferentiated stem cell into a specific lineage. Accordingly, to prove that amniotic fluid samples indeed contain pluripotent stem cells, it was essential to show that starting from one single stem cell differentiation into different cell lineages can be induced. For AFS cells, this was proven in three steps.

In 2006, Tsai and colleagues performed minimal dilution experiments to a single cell level, without prior marker selection. The so obtained clonal human AFS cell lines expressed the stem cell markers NANOG and Oct-4 and could efficiently be cultivated in vitro. Most importantly, these colleagues demonstrated that starting from one single AFS cell adipogenic, osteogenic, and neurogenic differentiations could be induced (Tsai et al. 2006).

In 2007, Atala's group performed immunoselection experiments to isolate CD117 (c-Kit)-positive human AFS cells. The so obtained clonal lines were Oct-4 positive and could be expanded in culture as stable lines with high proliferative capacity. Unlike embryonic stem cells, these AFS cells did not induce tumour formation in severe combined immunodeficient mice. Using marking of the cells via retroviral vector integration, the authors demonstrated that descending from a single cell, differentiation along six distinct lineages (adipogenic, osteogenic, myogenic, endothelial, neurogenic, and hepatic) could be induced (De Coppi et al. 2007).

Pluripotency in ES cells is also proven by their ability to form embryoid bodies. ES cells, when cultured without anti-differentiation factors under conditions in which they are unable to attach to the surface of culture dishes and without contact to feeder cells, can spontaneously form such three-dimensional multicellular aggregates. In 2010, it was reported that starting from a single human CD117-selected and Oct-4-positive AFS cell, embryoid bodies can be formed. This formation is accompanied by a decrease of stem cell marker expression and by the induction of differentiation into different lineages. This potential to form embryoid bodies is the ultimate proof of AFS cells to be pluripotent and now allows the recapitulation and investigation of the three-dimensional structure and tissue level contexts of many differentiation phenomenons (Valli et al. 2010).

Neurogenic differentiation of AFS cells

The usage of stem cells is a promising strategy for the treatment of neurodegenerative disorders and central nervous system injuries. Neuronal cells for therapy can be obtained via differentiation of ES cells, with the disadvantages of high tumour risk and ethical controversies, or of iPS cells, after exogenic induction of pluripotency. The latter are likely harbouring epigenetic memory and somatic mutations of the source cells. The subventricular zone and the hippocampus are examples of adult regions of the brain containing adult neuronal stem cells. However, these sources have an ethical limitation as well as a low proliferation capacity (reviewed in Koch et al. 2009; Mattis and Svendsen 2011). Accordingly, it is of great interest to investigate the putative neurogenic potential of AFS cells.

First evidence that amniotic fluid contains cells harbouring the potential of neurogenic differentiation was provided in 2004. Using a specific DMSO-containing low-serum differentiation medium, it was possible to induce neurogenic differentiation (proven by the detection of the typical morphological cell appearance and the induction of a variety of different neuronal and oligodendrocyte-specific markers) in many independent native human amniotic fluid cell samples (Prusa et al. 2004). Another study used a low serum medium with β-mercaptoethanol and basic fibroblast growth factor (FGF) and detected neurogenic differentiation of amniotic fluid cells via the analyses of morphological changes and the induction of class III β-tubulin (Tsai et al. 2004). Neurogenic differentiation of amniotic fluid cells detected by the induction of Neu N expression was also triggered with a combination of DMSO, basic FGF, and EGF. Interestingly, these authors studied amniotic fluid at time of birth rather than obtained from amniocenteses (Kim et al. 2007).

The first demonstration of neurogenic differentiation of AFS cells isolated at the single-cell level was published in 2006. Upon dopaminergic induction, extracts of these clonal AFS cells even showed evidence of dopamine release (Tsai et al. 2006). Monoclonal CD117+/Oct-4+ AFS cells were induced to differentiate upon the neurogenic lineage as proven by specific marker expression, morphology changes, detecting a barium-sensitive potassium channel via voltage clamping of individual cells, and by the study of neurotransmitter L-glutamate secretion. Although this is an impressive demonstration of the neurogenic/neuroectodermal differentiation potential of AFS cells, the authors note that additional work will be

necessary to prove that AFS cells are able to form mature neurons (De Coppi et al. 2007; Toselli et al. 2008).

To the best of our knowledge, the proof that AFS cells can differentiate to mature fully functional neurons is still missing in the literature. So far, neurogenic differentiation of AFS cells has been demonstrated in mouse, pig, and human (Mareschi et al. 2009; Phermthai et al. 2010; Jezierski et al. 2010; Zheng et al. 2010). It has been suggested that the efficiency of neurogenic differentiation depends on extracellular growth factors (Orciani et al. 2008, 2011). Human AFS cells express neuronal characteristics upon adenoviral transformation (Arnhold et al. 2008), and murine AFS cells maintain neurogenic differentiation potential upon baculovirus transduction (Liu et al. 2009). However, until now no report has detailedly checked whether mature neurons can be developed from AFS cells. Even the question whether dopaminergic neurogenic differentiation can be induced is a matter of discussion as data published are supporting this notion (Tsai et al. 2006; Pfeiffer and McLaughlin 2010), and other results provide evidence that human AFS cells do not differentiate into dopaminergic neurons (Donaldson et al. 2009).

In vivo nerve regeneration

In parallel to the in vitro investigations described above, several studies already analysed the potential of amniotic fluid cells in peripheral nerve regeneration in vivo. In the past, different approaches have been suggested to have beneficial effects on peripheral nerve regeneration, such as the administration of neurotrophic factors or electric field applications. However, currently, the field focuses on cell therapeutic approaches. Transplantation of ES cells and adult stem cells, including neural stem cells and mesenchymal stem cells, have been demonstrated to exert beneficial effects on peripheral nerve regeneration (Deumens et al. 2010; Dong and Yi 2010; Mattis and Svendsen 2011).

One group has intensively studied the potential of amniotic fluid cells for nerve regeneration using an injured sciatic nerve rat model. The authors create a 5-mm nerve defect by crushing the sciatic nerve using a vessel clamp. To evaluate putative beneficial effects of transplantation of amniotic fluid cells to the injured nerve, ankle kinematics, sciatic function index, and electrophysiology studies were performed. The authors reported that amniotic fluid cells can augment the growth of the injured nerve across the nerve gap and suggested this to be due to neurotrophic factors secreted by the amniotic fluid cells or to interacting effects with Schwann cells. They observed increased nerve myelination and improved motor function upon amniotic fluid cell transplantation (Pan et al. 2006, 2007, 2009; Cheng et al. 2010). Although this is a very important demonstration of a putative potential for nerve regeneration found in amniotic fluid cell samples, two major aspects must be clarified. Since the authors always used non-selected mixtures of amniotic fluid cells, without prior minimal dilution to a single-cell level, it remains to be answered whether one (or more) specific differentiated cell type already included in the native samples mediates these effects or whether (stem) cell differentiation occurs. Second, the underlying molecular mechanism of the observed beneficial effects must be clarified, focusing on the question whether the cells become part of the organic structure or just release factors with supportive effects. The same is true for a study showing that amniotic fluid cell transplantation can reverse the behavioural deficits in mice induced by ischaemic stroke (cerebral injury) (Rehni et al. 2007).

Besides these reports, in vivo data with amniotic fluid cells are rare. To investigate whether transplanted monoclonal c-Kit+/Oct-4+ human AFS cells can develop to dopaminergic neurons, colleagues performed rat brain implantation experiments. Integrated cells exhibited an immature morphology, expressing markers of neural progenitors and of neurons, but not of dopaminergic neurons, and only survived for a short time period (Donaldson et al. 2009). It has also been shown that transplanted monoclonal human c-Kit+/Oct-4+ human AFS can be detected within the choroid plexus and in close proximity to the ventricles of mouse brains. Further studies are warranted to clarify whether so transplanted cells really integrate and form functional neurons (De Coppi et al. 2007; Toselli et al. 2008).

Different stem cell types in native amniotic fluid?

The vast majority of studies performed to analyse the differentiation potential of monoclonal AFS cells were performed with cells, which express Oct-4 and c-Kit (CD117). These AFS cells were demonstrated to also be positive for CD44, CD73, CD90, CD105, and CD166 and negative for CD34, CD45, and CD133 (De Coppi et al. 2007; Siegel et al. 2008). On the other hand, CD133-positive cells with neurogenic differentiation potential have also been detected in human amniotic fluid (Prusa et al. 2004). These data could lead to the speculation that more than one stem cell type exist in human amniotic fluid. Considering the wide spectrum of cells of different origins within human amniotic fluid, this assumption would not be surprising. Further support for this notion comes from a recent report describing a detailed analysis of the differentiation potential of the CD117-positive compared to the CD117-negative cells contained in human amniotic fluid. In spite of the

CD117+ cells with higher efficiency, both cell populations harbour osteogenic and chondrogenic differentiation potentials. Adipogenic potential was only found in the CD117+ population. Interestingly, the CD117− populations showed a higher neuronal differentiation potential than the CD117+ cells although the latter population contains the Oct-4+/CD117+ stem cell type analysed in the majority of published reports using monoclonal AFS cells (Arnhold et al. 2010). These data provide additional evidence, supporting the theory of more than one stem cell type in amniotic fluid.

Future perspectives: basic science and clinical applications

In terms of basic research approaches, stem cells are optimal models for the detailed investigation of cell differentiation processes and their deregulation during disease development. AFS cells obviously represent an intermediate stage between embryonic stem cells and lineage-restricted adult progenitor cells. They might have advantages over embryonic stem cells, such as a lower risk for tumour development and no associated ethical concerns, as well as over adult stem cells, such as the higher proliferation rate and the higher differentiation potential. Especially for profound investigations of specific cell biological question, AFS cells might even have advantages over iPS cells, such as chromosomal stability, no need for exogenic induction of pluripotency, and no memory of the epigenetic code and somatic mutations of the source cells. The finding that clonal stem cell lines with pluripotent differentiation potential can be derived from human amniotic fluid and can be maintained in culture for over 250 population doublings while they retain genome stability raises possibilities for exciting new basic research approaches (Siegel et al. 2007; Pappa and Anagnou 2009; Dobreva et al. 2010; Mattis and Svendsen 2011). A major aim for the future could be to establish a biobank of monoclonal AFS cell lines generated from a wide variety of pregnancies with specific monogenetic diseases and chromosomal aberrations. So obtained AFS cell lines could allow the investigation of the consequences of naturally occurring disease-causing mutations for the molecular regulation of the different stem cell differentiation processes (Prusa et al. 2003b; Siegel et al. 2007). As the result of intensive and detailed technical investigations, a straightforward, efficient and highly reproducible protocol for siRNA-mediated long-term knock-down of endogenous gene functions in AFS cells has been published recently. This experimental innovation now allows the detailed investigation of the role of endogenous gene products for the variety of AFS cell differentiation processes (Rosner et al. 2010).

An important aspect that should be investigated in more detail in future is the question of the origin of AFS cells. Although the AFS cells were discovered 8 years ago and many speculations have been published, the origin of AFS cells is still as unclear as their in vivo function in amniotic fluid (Siegel et al. 2007, 2008; Pappa and Anagnou 2009; Dobreva et al. 2010).

The central nervous system comprises many cell types with different functions and different potentials, some of which might never survive when the cells are taken out of their endogenous settings. In addition, differentiation protocols do not exist for the in vitro generation of all these cell types. Furthermore, the methods for the induction of neurogenic differentiation vary between laboratories, including adherent monolayer cultures, stromal feeder layer cultivation, or embryoid body formation (Mattis and Svendsen 2011). With regard to the topic of this review, it would be of great interest to find out what kind of neurogenic cell types can be developed from AFS cells. However, before that, it would be most important to clarify whether AFS cells can differentiate into functional mature neurons, which could be proven by the analysis of the ability to fire tetrodotoxin-sensitive action potentials with the characteristic shape and duration, or by the demonstration of synapses by electron microscopy (Toselli et al. 2008).

The development of new stem-based therapies for heretofore incurable central nervous system pathologies, such as Parkinson's disease, spinal cord injury, multiple sclerosis or brain stroke is of great interest. Neural stem cells, which could be investigated for this purpose, can be found in the adult central nervous system and in the developing embryo, but these tissues are not easy available and raise ethical concerns (Deumens et al. 2010; Dong and Yi 2010; Mattis and Svendsen 2011). Currently, AFS cells become increasingly accepted as a new powerful tool for cell therapeutic approaches. In future, AFS cells could probably indeed provide a new source for the development of new approaches for stem cell therapies and tissue engineering. However, as already mentioned above, with respect to the topic of this review, the proof that AFS really can form mature neurons is essential before further project into the direction of clinical applications can be considered. An interesting idea at least for the future will also be to biobank AFS cells from amniocentesis, with and without diagnosed genetic aberrations, for both basic research and translational medicine approaches (Siegel et al. 2008; Pappa and Anagnou 2009; Dobreva et al. 2010; Antonucci et al. 2010).

Acknowledgments Research in our laboratory is supported via grants from the Österreichische Nationalbank.

References

Antonucci I, Stuppia L, Kaneko Y, Yu S, Tajiri N, Bae EC, Chheda SH, Weinbren NL, Borlongan CV (2010) Amniotic fluid as rich source of mesenchymal stromal cells for transplantation therapy. Cell Transplant. doi:10.3727/096368910X539074

Arnhold S, Post C, Glüer S, Hoopmann M, Wenisch S, Volpers C, Addicks K (2008) Neuronal characteristics of amniotic fluid derived cells after adenoviral transformation. Cell Biol Int 32:1559–1566

Arnhold S, Glüer S, Hartmann K, Raabe O, Addicks K, Wenisch S, Hoopmann M (2010) Amniotic-fluid stem cells: growth dynamics and differentiation potential after a CD-117-based selection procedure. Stem Cell Int 2011:1–12

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

Cheng F-C, Tai M-H, Sheu M-L, Chen C-J, Yang D-Y, Su H-L, Ho S-P, Lai S-Z, Pan H-C (2010) Enhancement of regeneration with glia cell line-derived neurotrophic factor-transduced human amniotic fluid mesenchymal stem cells after sciatic nerve crush injury. J Neurosurg 112:868–879

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

Deumens R, Bozkurt A, Meek MF, Marcus MA, Joosten EA, Weis J, Brook GA (2010) Repairing injured peripheral nerves: bridging the gap. Prog Neurobiol 92:245–276

Ditadi A, de Coppi P, Picone O, Gautreau L, Smati R, Six E, Bonhomme D, Ezine S, Frydman R, Cavazzana-Calvo M, Andre-Schmutz I (2009) Human and murine amniotic c-Kit+Lin− cells display hematopoietic activity. Blood 113: 3953–3960

Dobreva P, Pereira PNG, Deprest J, Zwijsen A (2010) On the origin of amniotic stem cells: of mice and men. Int J Dev Biol 54:761–777

Donaldson AE, Cai J, Yang M, Iacovitti L (2009) Human amniotic fluid stem cells do not differentiate into dopamine neurons in vitro or after transplantation in vivo. Stem Cells Dev 18:1003–1011

Dong MM, Yi TH (2010) Stem cell and peripheral nerve injury and repair. Facial Plast Surg 26:421–427

Hauser PV, DeFazio R, Bruno S, Sdei S, Grange C, Bussolati B, Benedetto C, Camussi G (2010) Stem cells derived from human amniotic fluid contribute to acute kidney injury recovery. Am J Pathol 177:2011–2021

In 't Anker PS, Scherjon SA, van der Kleijburg-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

Jezierski A, Gruslin A, Tremblay R, Ly D, Smith C, Turksen K, Sikorska M, Bani-Yaghoub M (2010) Probing stemness and neural commitment in human amniotic fluid cells. Stem Cell Rev Rep 6:199–214

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

Kim J, Lee Y, Kim H, Hwang KJ, Kwon HC, Kim SK, Cho DJ, Kang SG, You J (2007) Human amniotic fluid-derived stem cells have characteristics of multipotent stem cells. Cell Prolif 40:75–90

Koch P, Kokaia Z, Lindvall O, Brüstle O (2009) Emerging concepts in neural stem cell research: autologous repair and cell-based disease modelling. Lancet Neurol 8:819–829

Kolambkar YM, Peister A, Soker S, Atala A, Guldberg RE (2007) Chondrogenic differentiation of amniotic fluid-derived stem cells. J Mol Hist 38:405–413

Liu Z-S, Xu Y-F, Feng S-W, Li Y, Yao X-L, Lu X-L, Zhang C (2009) Baculovirus-transduced mouse amniotic fluid-derived stem cells maintain differentiation potential. Ann Hematol 88:565–572

Mareschi K, Rustichelli D, Comunanza V, DeFazio R, Cravero C, Morterra G, Martinoglio B, Medico E, Carbone E, Benedetto C, Fagioli F (2009) Multipotent mesenchymal stem cells from amniotic fluid originate neural precursors with functional voltage-gated sodium channels. Cytotherapy 11:534–538

Mattis VB, Svendsen CN (2011) Induced pluripotent stem cells: a new revolution for clinical neurology? Lancet Neurol 10:383–394

Orciani M, Emanuelli M, Martino C, Pugnaloni A, Tranquilli AL, DiPrimio R (2008) Potential role of culture mediums for successful isolation and neuronal differentiation of amniotic fluid stem cells. Int J Immunopathol Pharmacol 21:595–602

Orciani M, Morabito C, Emanuelli M, Guarnieri S, Sartini D, Giannubilo SR, DiPrimio R, Tranquilli AL, Mariggio MA (2011) Neurogenic potential of mesenchymal-like stem cells from human amniotic fluid: the influence of extracellular growth factors. J Biol Regul Homeost Agents 25:115–130

Pan H-C, Yang D-Y, Chiu Y-T, Lai S-Z, Wang Y-C, Chang M-H, Cheng F-C (2006) Enhanced regeneration in injured sciatic nerve by human amniotic mesenchymal stem cells. J Clin Neurosci 13:570–575

Pan H-C, Cheng F-C, Chen C-J, Lai S-Z, Lee C-W, Yang D-Y, Chang M-H, Ho S-P (2007) Post-injury in rat sciatic nerve facilitated by neurotrophic factors secreted by amniotic fluid mesenchymal stem cells. J Clin Neurosci 14:1089–1098

Pan H-C, Chin C-S, Yang D-Y, Ho S-P, Chen C-J, Hwang S-M, Chang M-H, Cheng F-C (2009) Human amniotic fluid mesenchymal stem cells in combination with hyperbaric oxygen augment peripheral nerve regeneration. Neurochem Res 34:1304–1316

Pappa KI, Anagnou NP (2009) Novel sources of fetal stem cells: where do they fit on the developmental continuum? Regen Med 4:423–433

Perin L, Giuliani S, Jin D, Sedrakyan S, Carraro G, Habibian R, Warburton D, Atala A, De Filippo RE (2007) Renal differentiation of amniotic fluid stem cells. Cell Prolif 40:936–948

Perin L, Sedrakyan S, Giuliani S, DaSacco S, Carraro G, Shiri L, Lemley KV, Rosol M, Wu S, Atala A, Warburton D, DeFilippo RE (2010) Protective effect of human amniotic fluid stem cells in an immunodeficient mouse model of acute tubular necrosis. Plos One 5:1–16

Pfeiffer S, McLaughlin D (2010) In vitro differentiation of human amniotic fluid-derived cells: augmentation towards a neuronal dopaminergic phenotype. Cell Biol Int 34:959–967

Phermthai T, Odglun Y, Julavijitphong S, Titapant V, Chuenwattana P, Vantanasiri C, Pattanapanyasat K (2010) A novel method to derive amniotic fluid stem cells for therapeutic purposes. BMC Cell Biol 11:79–88

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 (2003a) 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, Freilinger A, Bernachek G, Hengstschläger M (2003b) Stem cell marker expression in

human trisomy 21 amniotic fluid cells and trophoblasts. J Neural Transm 67:235–242

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

Rehni AK, Singh N, Jaggi AS, Singh M (2007) Amniotic fluid derived stem cells ameliorate focal cerebral ischaemia-reperfusion injury induced behavioural deficits in mice. Behav Brain Res 183:95–100

Rosner M, Siegel N, Fuchs C, Slabina N, Dolznig H, Hengstschläger M (2010) Efficient siRNA-mediated prolonged gene silencing in human amniotic fluid stem cells. Nat Protoc 5:1081–1095

Siegel N, Rosner M, Hanneder M, Valli A, Hengstschläger M (2007) Stem cells in amniotic fluid as new tools to study human genetic diseases. Stem Cell Rev 3:256–264

Siegel N, Rosner M, Hanneder M, Freilinger A, Hengstschläger M (2008) Human amniotic fluid stem cells: a new perspective. Amino Acids 35:291–293

Siegel N, Valli A, Fuchs C, Rosner M, Hengstschläger M (2009) Induction of mesenchymal/epithelial marker expression in human amniotic fluid stem cells. RBM Online 19:838–846

Siegel N, Rosner M, Unbekandt M, Fuchs C, Slabina N, Dolznig H, Davies JA, Lubec G, Hengstschläger M (2010) Contribution of human amniotic fluid stem cells to renal tissue formation depends on mTOR. Hum Mol Genet 19:3320–3331

Toselli M, Cerbai E, Rossi F, Cattaneo E (2008) Do amniotic fluid-derived stem cells differentiate into neurons in vitro? Nat Biotechnol 26:269–271

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

Tsai M-S, Hwang S-M, Tsai Y-L, Cheng F-C, Lee J-L, Chang Y-J (2006) Clonal amniotic fluid-derived stem cells express characteristics of both mesenchymal and neural stem cells. Biol Reprod 74:545–551

Valli A, Rosner M, Fuchs C, Siegel N, Bishop CE, Dolznig H, Mädel U, Feichtinger W, Atala A, Hengstschläger M (2010) Embryoid body formation of human amniotic fluid stem cells depends on mTOR. Oncogene 29:966–977

Zheng Y-M, Zhao X-E, An Z-X (2010) Neurogenic differentiation of EGFP gene transfected amniotic fluid-derived stem cells from pigs at intermediate and late gestational ages. Reprod Dom Anim 45:78–82