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A Paediatric Perspective on Stem Cells: Expression, Function, and Clinical Relevance (Stem Cells from Amniotic Fluid)

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Abstract

In this paper, we provide an overview of the potential advantages and disadvantages of different stem and progenitor cell populations identified to date in amniotic fluid, along with their properties and potential clinical applications.

Keywords: Stem cells; Expression; Function; Clinical relevance; Amniotic fluid

Introduction

In the last ten years, placenta, fetal membranes (i.e. amnion and chorion), and amniotic fluid have been extensively investigated as a potential non-controversial source of stem cells. They are usually discarded after delivery and are accessible during pregnancy through amniocentesis and chorionic villus sampling [1]. Several populations of cells with multilineage differentiation potential and immunomodulatory properties have been isolated from the human placenta and fetal membranes; they have been classified by an international workshop [2] as human amniotic epithelial cells (hAECs) [3-8] human amniotic mesenchymal stromal cells (hAMSCs) [9,10], human chorionic mesenchymal stromal cells (hCMSCs) and human chorionic trophoblastic cells (hCTCs). In the amniotic fluid (AF), two main populations of stem cells have been isolated so far:

1. Amniotic fluid mesenchymal stem cells (AFMSCs)

2. Amniotic fluid stems (AFS) cells. Although only recently described, these cells may, given the easier accessibility of the AF in comparison to other extra-embryonic tissues, hold much promise in regenerative medicine.

Amniotic Fluid: Function, Origin, and Composition

The AF is the clear, watery liquid that surrounds the growing fetus within the amniotic cavity. It allows the fetus to freely grow and move inside the uterus, protects it from outside injuries by cushioning sudden blows or movements by maintaining consistent pressure and temperature, and acts as a vehicle for the exchange of body chemicals with the mother [11,12].

In humans, the AF starts to appear at the beginning of the second week of gestation as a small film of liquid between the cells of the epiblast. Between days 8 and 10 after fertilization, this fluid gradually expands and separates the epiblast (i.e. the future embryo) from the amnio- blasts (i.e. the future amnion), thus forming the amniotic cavity [3-8]. Thereafter, it progressively increases in volume, completely surrounding the embryo after the fourth week of pregnancy.

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20 ml in the seventh week to 600 ml in the 25th week, 1,000 ml in the 34th week, and 800 ml at birth. During the first half of gestation, the AF results from active sodium and chloride transport across the amniotic membrane and the non-keratinized fetal skin, with concomitant passive movement of water [13]. In the second half of gestation, the AF is constituted by fetal urine, gastrointestinal excretions, respiratory secretions, and substances exchanged through the sac membranes [14-16].

The AF is primarily composed of water and electrolytes (98-99%) but also contains chemical substances (e.g. glucose, lipids, proteins, hormones, and enzymes), suspended materials (e.g. vernix caseosa, lanugo hair, and meconium), and cells. AF cells derive both from extra- embryonic structures (i.e. placenta and fetal membranes) and from embryonic and fetal tissues [17,18]. Although AF cells are known to express markers of all three germ layers Cremer et al. [20] their exact origin still represents a matter of discussion; the consensus is that they mainly consist of cells shed in the amniotic cavity from the developing skin, respiratory apparatus, and urinary and gastrointestinal tracts [19-21]. AF cells display a broad range of morphologies and behaviors varying with gestational age and fetal development [22]. In normal conditions, the number of AF cells increases with advancing gestation; if a fetal disease is present, AF cell counts can be either dramatically reduced (e.g. intrauterine death, urogenital atresia) or abnormally elevated (e.g. anencephaly, spina bifida, exomphalos [23,24]. Based on their morphological and growth characteristics, viable adherent cells from the AF are classified into three main groups: epithelioid (33.7%), amniotic fluid (60.8%), and fibroblastic type (5.5%) [25]. In the event of fetal abnormalities, other types of cells can be found in the AF, e.g. neural cells in the presence of neural tube defects and peritoneal cells in the case of abdominal wall malformations [26-28].

The majority of cells present in the AF is terminally differentiated and has limited prolif- erative capabilities [29,30]. In the 1990s, however, two groups demonstrated the presence in the AF of small subsets of cells harboring a proliferation and differentiation potential.

First, Torricelli reported the presence of hematopoietic progenitors in the AF collected before the 12th week of gestation [31]. Then Streubel was able to differentiate AF cells into myocytes, thus suggesting the presence in the AF of non- hematopoietic precursorsm [32]. These results initiated a new interest in the AF as an alternative source of cells for therapeutic applications.

Amniotic Fluid Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) represent a population of multipotent stem cells able to differentiate towards mesoderm-derived lineages (i.e. adipogenic, chondrogenic, myogenic, and osteogenic) [33]. Initially identified in adult bone marrow, where they represent 0.001e0.01% of total nucleated cells [34], MSCs have since been isolated from several adult (e.g. adipose tissue, skeletal muscle, liver, brain), fetal (i.e. bone marrow, liver, blood), and extra-embryonic tissues (i.e. placenta, amnion) [35].

The presence of a subpopulation of AF cells with mesenchymal features, able to proliferate in vitro more rapidly than comparable fetal and adult cells, was described for the first time in 2001 [36]. In 2003, In't Anker demonstrated that the AF can be an abundant source of fetal cells that exhibit a phenotype and a multilineage differentiation potential similar to that of bone marrow-derived MSCs; these cells were named AF mesenchymal stem cells (AFMSCs). Soon after this paper, other groups independently confirmed similar results.

Isolation and Culture

AFMSCs can be easily obtained: in humans, from small volumes (2e5 ml) of second and third trimester AF [37,38], where their percentage is estimated to be 0.9e1.5% of the total AF cells [39], and in rodents, from the AF collected during the second or third week of pregnancy [40,41]. Various protocols have been proposed for their isolation; all are based on the expansion of unselected populations of AF cells in serum-rich conditions without feeder layers, allowing cell selection by culture conditions. The success rate of the isolation of AFMSCs is reported by different authors to be 100% [42,43]. AFMSCs grow in basic medium containing fetal bovine serum (20%) and fibroblast growth factor (5 ng/ml). Importantly, it has been very recently shown that human AFMSCs can be also cultured in the absence of animal serum without losing their properties [44]; this finding is a fundamental prerequisite for the beginning of clinical trials in humans.

Characterization

The fetal versus maternal origin of AFMSCs has been investigated by different authors. Molecular HLA typing and amplification of the SRY gene in AF samples collected from male fetuses [45] demonstrated the exclusive fetal derivation of these cells. However, whether AFMSCs originate from the fetus or from the fetal portion of extra-embryonic tissues is still a matter of debate [46]. AFMSCs display a uniform spindle-shaped fibroblast-like morphology similar to that of other MSC populations and expand rapidly in culture [47]. Human cells derived from a single 2 ml AF sample can increase up to 180-106 cells within four weeks (three passages) and, as demonstrated by growth kinetics assays, possess a greater proliferative potential (average doubling time 25e38 hours) in comparison to that of bone marrow-derived MSCs (average doubling time 30e90 hours) [48-50]. Moreover, AFMSCs' clonogenic potential has been proved to exceed that of MSCs isolated from bone marrow (86 - 4.3 vs. 70 - 5.1 colonies) [51]. Despite their high proliferation rate, AFMSCs retain a normal karyotype and do not display tumorigenic potential even after extensive expansion in culture [52,53].

Analysis of AFMSC transcriptome demonstrated that: (1) AFMSCs' gene expression profile, as well as that of other MSC populations, remains stable between passages in culture, enduring cryopreservation and thawing well; (2) AFMSCs share with MSCs derived from other sources a core set of genes involved in extracellular matrix remodeling, cytoskeletal organization, chemokine regulation, plasmin activation, TGF-b and Wnt signaling pathways; (3) in comparison to other MSCs, AFMSCs show a unique gene expression signature that consists of the up-regulation of genes involved in signal transduction pathways (e.g. HHAT, F2R, F2RL) and in uterine maturation and contraction (e.g. OXTR, PLA2G10), thus suggesting a role of AFMSCs in modulating the interactions between the fetus and the uterus during pregnancy [54].

The cell-surface antigenic profile of human AFMSCs has been determined through flow cytometry by different investigators. Cultured human AFMSCs are positive for mesenchymal markers (i.e. CD90, CD73, CD105, CD166), for several adhesion molecules (i.e. CD29, CD44, CD49e, CD54), and for antigens belonging to the major histocompatibility complex I (MHC-I). They are negative for hematopoietic and endothelial markers (e.g. CD45, CD34, CD14, CD133, CD31). AFMSCs exhibit a broad differentiation potential towards mesenchymal lineages. Under specific in vitro inducing conditions, they are able to differentiate towards the adipogenic, osteogenic, and chondrogenic lineage.

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Immunophenotype of Culture-expanded Second and Third Trimester Human AFMSC: Results by Different Groups: Markers, Mesenchymal, Endotelial and hematopoietic, Integrins, Selectins Ig-superfamily. Despite not being pluripotent, AFMSCs can be efficiently reprogrammed into pluripotent stem cells (iPS) via retroviral transduction of defined transcription factors (Oct4, Sox2, Klf-4, c-Myc). Strikingly, AFMSC reprogramming capacity is significantly higher (100-fold) and much quicker (6 days vs. 16e30 days) in comparison to that of somatic cells such as skin fibroblasts. As iPS derived from adult cells, AF-derived iPS generate embryoid bodies (EBs) and differ- entiate towards all three germ layers in vitro, and *in vivo* form teratomas when injected into SCID mice [54].

Preclinical Studies

After AFMSC identification, various studies investigated their therapeutic potential in different experimental settings. Different groups demonstrated that AFMSCs are able not only to express cardiac and endothelial specific markers under specific culture conditions, but also to integrate into normal and ischemic cardiac tissue, where they differentiate into cardiomyocytes and endothelial cells [55-58]. In a rat model of bladder cryo-injury, AFMSCs show the ability to differentiate into smooth muscle and to prevent the compensatory hypertrophy of surviving smooth muscle cells [59].

AFMSCs can be a suitable cell source for tissue engineering of congenital malformations. In an ovine model of diaphragmatic hernia, repair of the muscle deficit using grafts engineered with autologous mesenchymal amniocytes leads to better structural and functional results in comparison to equivalent fetal myoblast-based and acellular implants [60]. Engineered cartilaginous grafts have been derived from

AFMSCs grown on biodegradable meshes in serum-free chondrogenic conditions for at least 12 weeks; these grafts have been successfully used to repair tracheal defects in foetal lambs when implanted in utero [61]. The surgical implantation of AFMSCs seeded on nanofibrous.

Stem Cells from Amniotic Fluid

Scaffolds and pre differentiated in vitro towards the osteogenic lineage into a leporine model of sternal defect leads to a complete bone repair in 2 months' time [62]. Intriguingly, recent studies suggest that AFMSCs can harbor trophic and protective effects in the central and peripheral nervous systems. Pan showed that AFMSCs facilitate peripheral nerve regeneration after injury and hypothesized that this can be determined by cell secretion of neurotrophic factors [63,64]. After transplantation into the striatum, AFMSCs are capable of surviving and integrating in the rat adult brain and migrating towards areas of ischemic damage [65]. Moreover, the intra- ventricular administration of AFMSCs in mice with focal cerebral ischemia-reperfusion injuries significantly reverses neurological deficits in the treated animals [66]. Remarkably, it has also been observed that AFMSCs present *in vitro* an immunosuppressive effect similar to that of bone marrow-derived MSCs [67]. Following stimu- lation of peripheral blood mononuclear cells with anti-CD3, anti-CD28, or phytohemagglu- tinin, irradiated AFMSCs determine a significant inhibition of T-cell proliferation with dose-dependent kinetics [68].

Amniotic Fluid Stem Cells

The first evidence that the AF could contain pluripotent stem cells was provided in 2003 when Prusa described the presence of a distinct subpopulation of proliferating AF cells (0.1- 0.5% of the cells present in the AF) expressing the pluripotency marker Oct4 at both transcriptional and proteic levels [69]. Oct 4 (i.e. octamer binding transcription factor 4) is a nuclear transcription factor that plays a critical role in maintaining ES cell differentiation potential and capacity of self-renewal [70-72]. Other than by ES cells, Oct4 is specifically expressed by germ cells, where its inacti- vation results in apoptosis, and by embryonal carcinoma cells and tumors of germ cell origin, where it acts as an oncogenic fate determinant [73-76]. While its role in stem cells of fetal origin has not been completely addressed, it has been recently demonstrated that Oct4 is neither expressed nor required by somatic stem cells or progenitors [77-79].

After Prusa, different groups confirmed the expression of Oct4 and of its transcriptional targets (e.g. Rex-1) in the AF [80,81]. Remarkably, Karlmark transfected human AF cells with the green fluorescent protein gene under either the Oct4 or the Rex-1 promoter and established that some AF cells were able to activate these promoters. Several authors subsequently reported the possibility of harvesting AF cells displaying features of pluripotent stem cells [82,83]. Thereafter, the presence of a cell population able to generate clonal cell lines capable of differentiating into lineages representative of all three embryonic germ layers was definitively demonstrated [84]. These cells, named AF stem (AFS) cells, are characterized by the expression of the surface antigen c-kit (CD117), which is the type III tyrosine kinase receptor of the stem cell factor [85].

Isolation and Culture

The proportion of c-kitþ cells in the amniotic fluid varies over the course of gestation, roughly describing a Gaussian curve; they appear at very early time points in gestation (i.e. at 7 weeks of amenorrhea in humans and at E9.5 in mice) and present a peak at midgestation equal to 90 - 104 cells/fetus at 20 weeks of pregnancy in humans and to 10,000 cells/fetus at E12.5 in mice [86]. Human AFS cells can be derived either from small volumes (5 ml) of second trimester AF (14e22 weeks of gestation) or from confluent back-up amniocentesis cultures. Murine AFS cells are obtainable from the AF collected during the second week of gestation (E11.5e14.5) [87-89].

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Cell isolation is based on a two-step protocol consisting of the prior immunological selection of c- kit positive cells from the AF (approximately 1% of total AF cells) and of the subsequent expansion of these cells in culture [90-95]. Isolated AFS cells can be expanded in feeder layer-free, serum-rich conditions without evidence of spontaneous differentiation in vitro. Cells are cultured in basic medium containing 15% of fetal bovine serum and Chang supplement.

Characterization

Karyotype analysis of human AFS cells deriving from pregnancies in which the fetus was male revealed the fetal origin of these cells [96]. AFS cells proliferate well during ex vivo expansion. When cultivated, they display a spectrum of morphologies ranging from a fibroblast-like to an oval-round shape. As demonstrated by different authors, AFS cells possess a great clonogenic potential [97,98]. Clonal AFS cell lines expand rapidly in culture (doubling time 1⁄4 36 h) and, more interestingly, maintain a constant telomere length (20 kbp) between early and late passages. Almost all clonal AFS cell lines express markers of a pluripotent undifferentiated state: Oct4 and NANOG [99-103]. However, they have been proved not to form tumors when injected in severe combined immunodeficient (SCID) mice [104]. The cell-surface antigenic profile of AFS cells has been determined through flow cytometry by different investigators. Cultured human AFS cells are positive for ES cell (e.g. SSEA-4) and mesenchymal markers (e.g. CD73, CD90, CD105), for several adhesion mole- cules (e.g. CD29, CD44), and for antigens belonging to the MHC-I. They are negative for hematopoietic and endothelial markers (e.g. CD14, CD34, CD45, CD133, and CD31) and for antigens belonging to the major histocompatibility complex II (MHC-II).

As stability of cell lines is a fundamental prerequisite for basic and translational research, AFS cell capacity of maintaining their baseline characteristics over passages has been evaluated based on multiple parameters. Despite their high proliferation rate, AFS cells and derived clonal lines show a homogeneous, diploid DNA content without evidence of chromosomal rearrangement even after expansion to 250 population doublings [105,106]. Moreover, AFS cells maintain constant morphology, doubling time, apoptosis rate, cell cycle distribution, and marker expression (e.g. Oct4, CD117, CD29, CD44) up to 25 passages. During *in vitro* expansion, (A) Human AFS cells mainly display a spindle-shaped morphology during *in vitro* cultivation under feeder layer-free, serum-rich conditions. (BeC) Clonal human AFS cell lines retain long telomeres and a normal karyotype after more than 250 cell divisions. (B) Conserved telomere length of AFS cells between early passage (20 population doublings, lane 3) and late passage (250 population doublings, lane 4). Short length (lane 1) and high length (lane 2) telomere standards provided in the assay kit. (C) Giemsa band karyogram showing chromosomes of late passage (250 population doublings) cells. However, cell volume tends to increase and significant fluctuations of proteins involved in different networks (i.e. signaling, antioxidant, proteasomal,

cytoskeleton, connective tissue, and chaperone proteins) can be observed using a gel-based proteomic approach [107]; the significance of these modifications warrants further investigations but needs to be taken into consideration when interpreting experiments run over several passages and comparing results from different groups.

AFS cells and, more importantly, derived clonal cell lines are able to differentiate towards tissues representative of all three embryonic germ layers, both spontaneously, when cultured in suspension to form EBs, and when grown in specific differentiation conditions.

EBs consists of three-dimensional aggregates of ES cells, which recapitulate the first steps of early mammalian embyogenesis [108-110]. As ES cells, when cultured in suspension and without anti-differentiation factors, AFS cells harbor the potential to form EBs with high efficiency: the incidence of EB formation (i.e. percentage of number of EB recovered from 15 hanging drops) is estimated to be around 28% for AFS cell lines and around 67% for AFS cell clonal lines. Similarly to ES cells, EB generation by AFS cells is regulated by the mTor (i.e. mammalian target of rapamycin) pathway and is accompanied by a decrease of Oct4 and Nodal expression and by an induction of endodermal (GATA4), mesodermal (Brachyury, HBE1), and ectodermal (Nestin, Pax6) markers [111,112].

In specific mesenchymal differentiation conditions, AFS cells express molecular markers of adipose, bone, muscle, and endothelial differentiated cells (e.g. LPL, desmin, osteocalcin, and V-CAM1). In the adipogenic, chondrogenic, and osteogenic medium, AFS cells respectively develop intracellular lipid droplets, secrete glycosaminoglycans, and produce mineralized calcium [113]. In conditions inducing cell differentiation towards the hepatic lineage, AFS cells express hepatocyte-specific transcripts (e.g. albumin, alpha-fetoprotein, multidrug resistance membrane transporter 1) and acquire the liver-specific function of urea secretion [114]. In neuronal conditions, AFS cells are capable of entering the neuroectodermal lineage. After induction, they express.

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AFS cells differentiation into lineages representative of the three embryonic germ layers. (A) Hepatogenic differentiation: urea secretion by human AFS cells before (rectangles) and after (diamonds) hepatogenic *in vitro* differentiation. (B) Neurogenic differentiation: secretion of neurotransmitter glutamic acid in response to potassium ions. (C) Osteogenic differentiation: mouse micro CT scan 18 weeks after implantation of printed constructs of engineered bone from human AFS cells; arrow head: region of implantation of control scaffold without AFS cells; rhombus: scaffolds seeded with AFS cells. Adapted from de Coppi et al. [114] neuronal markers (e.g. GIRK potassium channels) exhibit barium-sensitive potassium current, and release glutamate after stimulation. Ongoing studies are investigating AFS cell capacity to yield mature, functional neurons [115-118]. AFS cells can be easily manipulated in vitro. They can be transduced with viral vectors more efficiently than adult MSCs, and, after infection, maintain their antigenic profile and the ability to differentiate into different lineages [119]. AFS cells labeled with super- paramagnetic micrometer-sized iron oxide particles (MPIOs) retain their potency and can be non-invasively tracked by MRI for at least four weeks after injection *in vivo* [119].

Preclinical Studies

Despite the very recent identification of AFS cells, several reports have investigated their potential applications in different settings.

Bone

Critical-sized segmental bone defects are one of the most challenging problems faced by orthopedic surgeons. Autologous and heterologous bone grafting are limited respectively by the small amount of tissue available for transplantation and by high re-fracture rates. Tissue engineering strategies that combine biodegradable scaffolds with stem cells capable of osteogenesis have been indicated as promising alternatives to bone grafting however, bone regen- eration through cell-based therapies has been limited so far by the insufficient availability of osteogenic cells.

The potential of AFS cells to synthesize mineralized extracellular matrix within porous scaf- folds has been investigated by different groups. After exposure to osteogenic conditions in static two-dimensional cultures, AFS cells differentiate into functional osteoblasts (i.e. activate the expression of osteogenic genes such as Runx2, Osx, Bsp, Opn, and Ocn, and produce alkaline phosphatase) and form dense layers of mineralized matrix. As demonstrated by clonogenic mineralization assays, 85% of AFS cells versus 50% of MSCs are capable of forming osteogenic colonies. When seeded into three-dimensional biode- gradable scaffolds and stimulated by osteogenic supplements (i.e. rhBMP-7 or dexametha- sone), AFS cells remain highly viable up to several months in culture and produce extensive mineralization throughout the entire volume of the scaffold [80-110].

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In vivo, when subcutaneously injected into nude rodents, predifferentiated AFS cell-scaffold constructs are able to generate ectopic bone structures in four weeks' time. AFS cells embedded in scaffolds, however, are not able to mineralize *in vivo* at ectopic sites unless previously pre-differentiated in vitro. These studies demonstrate the potential of AFS cells to produce three-dimensional mineralized bioengineered constructs and suggest that AFS cells may be an effective cell source for functional repair of large bone defects. Further studies are needed to explore AFS cell estrogenic potential when injected into sites of bone injury [100-120].

Cartilage

Enhancing the regeneration potential of hyaline cartilage is one of the most significant chal- lenges for treating damaged cartilage. The capacity of AFS cells to differentiate into functional chondrocytes has been tested *in vitro*. Human AFS cells treated with TGF-b1 have been proven to produce significant amounts of cartilaginous matrix (i.e. sulfated glycosaminoglycans and type II collagen) both in pellet and alginate hydrogel cultures.

Skeletal Muscle

Stem cell therapy is an attractive method to treat muscular degenerative diseases because only a small number of cells, together with a stimulatory signal for expansion, are required to obtain a therapeutic effect. The identification of a stem cell population providing efficient muscle regeneration is critical for the progression of cell therapy for muscle diseases. AFS cell capacity of differentiating

into the myogenic lineage has recently started to be explored. Under the influence of specific induction media containing 5-Aza-20-deoxycytidine, AFS cells are able to express myogenic-associated markers such as Mrf4, Myo-D, and desmin 231 both at a molecular and proteic level. However, when transplanted undifferentiated into damaged skeletal muscles of SCID mice, despite displaying a good tissue engraftment AFS cells did not differentiate towards the myogenic lineage. Further studies are needed to confirm the results of this single report [121-130].

Heart

Cardiovascular diseases are the first cause of mortality in developed countries despite advances in pharmacological, interventional, and surgical therapies. Cell trans- plantation is an attractive strategy to replace endogenous cardiomyocytes lost by myocardial infarction. Fetal and neonatal cardiomyocites are the ideal cells for cardiac regeneration as they have been shown to integrate structurally and functionally into the myocardium after trans- plantation. However, their application is limited by the ethical restrictions involved in the use of fetal and neonatal cardiac tissues.

Chiavegato et al. [20] investigated human AFS cell plasticity towards the cardiac lineage. Undif- ferentiated AFS cells express cardiac transcription factors at a molecular level (i.e. Nkx2.5 and GATA-4 mRNA) but do not produce any myocardial differentiation marker. Under in vitro cardiovascular inducing conditions (i.e. co-culture with neonatal rat cardiomyocytes), AFS cells express differentiated cardiomyocyte markers such as cTnI, indicating that an in vitro cardiomyogenic-like medium can lead to a spontaneous differentiation of AFS cells into cardiomyocyte-like cells. *In vivo*, when xenotransplanted in the hearts of immunodeficient rats 20 minutes after creating a myocardial infarction, AFS cell differentiation capabilities were impaired by cell immune rejection. More recently, we have proved that we could activate the myocardial gene program in GFP-positive rat AFS (GFP-rAFS cells) [131-140].

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The differentiation attained via a paracrine/contact action was confirmed using immunofluorescence, RT-PCR, and single-cell electrophysiological tests. Moreover, despite only a small number of Endorem-labeled GFP-rAFS, cells acquired an endothelial or smooth muscle phenotype and to a lesser extent CMs in an allogeneic acute myocardial infarction (AMI) context, and there was still improvement of ejection fraction as measured by magnetic resonance imaging (MRI) three weeks after injection. This could be partially due to a paracrine action perhaps mediated by the secretion of thymosin b4.

Hematopoietic System

Hematopoietic stem cells (HSCs) lie at the top of hematopoietic ontogeny and, if engrafted in the right niche, can theoretically reconstitute the organism's entire blood supply. Thus, the generation of autologous HSCs from pluripotent, patient-specific stem cells offer real promise for cell-therapy of both genetic and malignant blood disorders.

The hematopoietic potential of c-kitþ hematopoietic lineage negative cells present in the amniotic fluid (AFKL cells) has been recently explored. *In vitro*, human and murine AFKL cells exhibit strong multilineage hematopoietic potential. Cultured in semisolid medium, these cells are able to generate erythroid, myeloid, and lymphoid colonies. Moreover, murine cells exhibit the same clonogenic potential (0.03%) as hematopoietic progenitors present in the liver at the same stage of development. *In vivo*, mouse AFKL cells (i.e. 2 - 104 cells intravenously injected) are able to generate all three hematopoietic lineages after primary and secondary transplantation into immune compromised hosts (i.e. sub lethally irradiated Rag-/- mice), demonstrating their ability to self-renew. These results clearly show that c-kitþ cells present in the amniotic fluid have true hematopoietic potential both in vitro and *in vivo* [100-144].

Kidney

The incidence and prevalence of end stage renal disease (ESRD) continues to increase worldwide. Although renal transplantation represents a good treatment option, the shortage of compatible organs remains a critical issue for patients affected by ESRD. Therefore, the possibility of developing stem cell-based therapies for both glomerular and tubular repair has received intensive investigation in recent years. Different stem cell types have shown some potential in the generation of functional nephrons (but the most appropriate cell type for transplantation is still to be established. The potential of AFS cells in contributing to kidney development has been recently explored. Using a mesenchymal/epithelial differentiation protocol previously applied to demonstrate the renal differentiation potential of kidney stem cells, Siegel demonstrated that AFS cells and clonal-derived cell lines can differentiate towards the renal lineage; AFS cells sequentially grown in a mesenchymal differentiation medium containing EGF and PDGF-BB, and in an epithelial differentiation medium containing HGF and FGF4, reduce the expression of pluripotency markers (i.e. Oct4 and c-Kit) and switch on the expression of epithelial (i.e. CD51, ZO-1) and podocyte markers (i.e. CD2AP, NPHS2). AFS cells have also been shown to contribute to the development of primordial kidney structures during in vitro organogenesis; undifferentiated human AFS cells injected into a mouse embryonic kidney cultured ex vivo are able to integrate in the renal tissue, participate in all steps of nephrogenesis, and express molecular markers of early kidney differentiation such as ZO-1, claudin, and GDNF. Finally, very recent *in vivo* experiments show that AFS cells directly injected into damaged kidneys are able to survive, integrate into tubular structures, express mature kidney markers, and restore renal

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These studies demonstrate the nephrogenic potential of AFS cells and warrant further investigation of their potential use for cell-based kidney therapies.

Lung

Chronic lung diseases are common and debilitating; medical therapies have restricted efficacy and lung transplantation is often the only effective treatment. The use of stem cells for lung repair and regeneration after injury holds promise as a potential therapeutic approach for many lung diseases; however, current studies are still in their infancy.

AFS cell ability to integrate into the lung and to differentiate into pulmonary lineages has been elegantly investigated in different experimental models of lung damage and development. In vitro, human AFS cells injected into mouse embryonic lung explants engraft into the epithe- lium and into the mesenchyme and express the early

pulmonary differentiation marker TFF1. *In vivo*, in the absence of lung damage, systemically administered AFS cells show the capacity to home to the lung but not to differentiate into specialized cells; while, in the presence of lung injury, AFS cells not only exhibit a strong tissue engraftment but also express specific alveolar and bronchiolar epithelial markers (e.g. TFF1, SPC, CC10). Remark- ably, cell fusion fenomena were elegantly excluded and long-term experiments confirmed the absence of tumor formation in the treated animals up to 7 months after AFS cell injection [100-144].

Intestine

To date, very few studies have considered the employment of stem cells in gastroenterological diseases. Although still at initial stages and associated with numerous problems, ever- increasing experimental evidence supports the intriguing hypothesis that stem cells may be possible candidates to treat and/or prevent intestinal diseases.

In a study evaluating AFS cell transplantation into healthy newborn rats, Ghionzoli demon- strated that, after intraperitoneal injection, AFS cells (1) diffuse systemically within a few hours from their administration in 90% of the animals, (2) engraft in several organs of the abdominal and thoracic compartment and (3) localize preferentially in the intestine colonizing the gut in 60% of the animals. Preliminary *in vivo* experiments investigating the role of AFS cells in a neonatal rat model of necrotizing enterocolitis show that intraperitoneal- injected AFS cells are able not only to integrate into all gut layers but also to reduce bowel damage, improve rat clinical status, and lengthen animal survival.

Conclusions

Many stem cell populations (e.g. embryonic, adult, and fetal stem cells) as well as methods for generating pluripotent cells (e.g. nuclear reprogramming) have been described to date. All of them carry specific advantages and disadvantages and, at present, it has yet to be established which type of stem cell represents the best candidate for cell therapy. However, although it is likely that one cell type may be better than another, depending on the clinical scenario, the recent discovery of easily accessible cells of fetal derivation, not burdened by ethical concerns, in the AF has the potential to open new horizons in regenerative medicine. Amniocentesis, in fact, is routinely performed for the antenatal diagnosis of genetic diseases and its safety has been established by several studies documenting an extremely low overall fetal loss rate (0.06e0.83%) related to this procedure. Moreover, stem cells can be obtained from AF samples without interfering with diagnostic procedures.

Two stem cell populations have been isolated from the AF so far (i.e. AFMSCs and AFS cells) and both can be used as primary (not transformed or immortalized) cells without further

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Technical manipulations AFMSCs exhibit typical MSC characteristics: fibroblastic-like morphology, clonogenic capacity, multilineage differentiation potential, immunosuppressive properties, and expression of a mesenchymal gene expression profile and of a mesenchymal set of surface antigens. However, ahead of other MSC sources, AFMSCs are easier to isolate and show better proliferation capacities. The harvest of bone marrow remains, in fact, a highly invasive and painful procedure, and the number, the proliferation, and the differentiation potential of these cells decline with increasing age. Similarly, UCB-derived MSCs exist at a low percentage and expand slowly in culture.

AFS cells, on the other hand, represent a novel class of pluripotent stem cells with intermediate characteristics between ES cells and AS cells. They express both embryonic and mesenchymal stem cell markers, are able to differentiate into lineages representative of all embryonic germ layers, and do not form tumors after implantation *in vivo*. However, AFS cells have only recently identified and many questions need to be answered concerning their origin, epigenetic state, immunological reactivity, and regeneration and differentiation potential *in vivo*. AFS cells, in fact, may not differentiate as promptly as ES cells and their lack of tumorigenesis can be argued against their pluripotency.

Although further studies are needed to better understand their biologic properties and to define their therapeutic potential, stem cells present in the AF appear to be promising candidates for cell therapy and tissue engineering. In particular, they represent an attractive source for the treatment of perinatal disorders such as congenital malformations (e.g. congenital diaphragmatic hernia) and acquired neonatal diseases requiring tissue repair/ regeneration (e.g. necrotizing enter colitis). In a future clinical scenario, AF cells collected during a routinely performed amniocentesis could be banked and, in case of need, subsequently expanded in culture or engineered in cellular grafts. In this way, affected children could benefit from having autologous expanded/engineered cells ready for implantation either before birth or in the neonatal period.

References

1. Alviano F, Fossati V, Marchionni C, Arpinati M, Bonsi L, et al. (2007) Term Amniotic membrane is a high throughput source for multipotent Mesenchymal Stem Cells with the ability to differentiate into endothelial cells in vitro. BMC Dev Biol 7: 11.
2. Andrade CF, Wong AP, Waddell TK, Keshavjee S, Liu M (2007) Cell-based tissue engineering for lung regeneration. Am J Physiol Lung Cell Mol Physiol 292: L510-518.
3. Aula P, von Koskull H, Teramo K, Karjalainen O, Virtanen I, et al. (1980) Glial origin of rapidly adhering amniotic fluid cells. Br Med J 281: 1456-1457.
4. Bajada S, Mazakova I, Richardson JB, Ashammakhi N (2008) Updates on stem cells and their applications in regenerative medicine. J Tissue Eng Regen Med 2: 169-183.
5. Beardi J, Hessmann M, Hansen M, Rommens PM (2008) Operative treatment of tibial shaft fractures: a comparison of different methods of primary stabilisation. Arch Orthop Trauma Surg 128: 709-715.
6. Berg JS, Goodell MA (2007) An argument against a role for Oct4 in somatic stem cells. Cell Stem Cell 1: 359-360.
7. Bianco P, Robey PG (2001) Stem cells in tissue engineering. Nature 414: 118e121.
8. Bollini S, Pozzobon M, Nobles M (2005) Amniotic fluid stem cells can acquire a cardiomyogenic phenotype in vitro but their survival in vivo is limited when used in an allogeneic setting.
9. Bossolasco P, Montemurro T, Cova L (2006) Molecular and phenotypic characterization of human AF cells and their differentiation potential. Cell Research 16: 329e336.
10. Brace RA, Resnik R (1999) Dynamics and disorders of amniotic fluid. In: Creasy RK, Renik R (eds.), Maternal Fetal Medicine. Philadelphia: Saunders, pp: 623-643.
11. Bruce SJ, Rea RW, Steptoe AL, Busslinger M, Bertram JF, et al. (2007) In vitro differentiation of murine embryonic stem cells toward a renal lineage. Differentiation 75: 337-349.

12. Bruno S, Bussolati B, Grange C, Collino F, di Cantogno LV, et al. (2009) Isolation and characterization of resident mesenchymal stem cells in human glomeruli. Stem Cells Dev 18: 867-880.
13. Bussolati B, Bruno S, Grange C, Buttiglieri S, Deregibus MC, et al. (2005) Isolation of renal progenitor cells from adult human kidney. Am J Pathol 166: 545-555.
14. Bussolati B, Hauser PV, Carvalhosa R, Camussi G (2009) Contribution of stem cells to kidney repair. Curr Stem Cell Res Ther 4: 2-8.
15. Cananzi M, Atala A, De Coppi P (2009) Stem cells derived from amniotic fluid: new potentials in regenerative medicine. Reprod Biomed Online 18 Suppl 1: 17-27.
16. Carraro G, Perin L, Sedrakyan S, Giuliani S, Tiozzo C, et al. (2008) Human amniotic fluid stem cells can integrate and differentiate into epithelial lung lineages. Stem Cells 26: 2902-2911.
17. Chambers I, Silva J, Colby D, Nichols J, Nijmeijer B, et al. (2007) Nanog safeguards pluripotency and mediates germline development. Nature 450: 1230-1234.
18. Chen WQ, Siegel N, Li L, Pollak A, Hengstschläger M, et al. (2009) Variations of protein levels in human amniotic fluid stem cells CD117/2 over passages 5-25. J Proteome Res 8: 5285-5295.
19. Chiavegato A, Bollini S, Pozzobon M (2007) Human AF-derived stem cells are rejected after trans- plantation in the myocardium of normal, ischemic, immuno-suppressed or immuno-deficient rat. Journal of Molecular and Cellular Cardiology 42: 746-759.
20. Cipriani S, Bonini D, Marchina E, Balgkouranidou I, Caimi L, et al. (2007) Mesenchymal cells from human amniotic fluid survive and migrate after transplantation into adult rat brain. Cell Biol Int 31: 845-850.
21. Caughey AB, Hopkins LM, Norton ME (2006) Chorionic villus sampling compared with amniocentesis and the difference in the rate of pregnancy loss. Obstet Gynecol 108: 612-616.
22. Cheng FC, Tai MH, Sheu ML (2009) Enhancement of regeneration with glia cell line-derived neurotrophic factor-transduced human amniotic fluid mesenchymal stem cells after sciatic nerve crush injury. Journal of Neurosurgery.
23. Cremer M, Treiss I, Cremer T (1981) Characterization of cells of AFs by immunological identification of intermediate-sized filaments: presence of cells of different tissue origin. Human Genetics 59: 373e379.
24. D'Ippolito G, Schiller PC, Ricordi C, Roos BA, Howard GA (1999) Age-related osteogenic potential of mesenchymal stromal stem cells from human vertebral bone marrow. J Bone Miner Res 14: 1115-1122.
25. Dai W, Kloner RA (2007) Myocardial regeneration by human amniotic fluid stem cells: challenges to be overcome. J Mol Cell Cardiol 42: 730-732.
26. Deans TL, Elisseeff JH (2009) Stem cells in musculoskeletal engineered tissue. Curr Opin Biotechnol 20: 537-544.
27. De Coppi, Callegari A, Chiavegato A (2007) AF and bone marrow derived mesenchymal stem cells can be converted to smooth muscle cells in the cryo-injured rat bladder and prevent compensatory hypertrophy of surviving smooth muscle cells. Journal of Urology 177: 369-376.
28. De Coppi P, Bartsch G Jr, Siddiqui MM, Xu T, Santos CC, et al. (2007) Isolation of amniotic stem cell lines with potential for therapy. Nat Biotechnol 25: 100-106.
29. Delo DM, Olson J, Baptista PM, D'Agostino RB Jr, Atala A, et al. (2008) Non-invasive longitudinal tracking of human amniotic fluid stem cells in the mouse heart. Stem Cells Dev 17: 1185-1194.
30. Ditadi A, de Coppi P, Picone O, Gautreau L, Smati R, et al. (2009) Human and murine amniotic fluid c-Kit+Lin- cells display hematopoietic activity. Blood 113: 3953-3960.
31. 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: 1003-1012.
32. Donovan PJ (2001) High Oct-ane fuel powers the stem cell. Nature Genetics 29: 246-247.
33. Eddleman KA, Malone FD, Sullivan L (2006) Pregnancy loss rates after midtrimester amniocentesis. Obstetrics and Gynecology 108: 1067-1072.
34. Farini A, Razini P, Erratico S (2009) Cell based therapy for Duchenne muscular dystrophy. Journal of Cell Physiology 221: 526-534.
35. Fauza D (2004) AF and placental stem cells. Best Practice and Research Clinical Obstetrics and Gynaecology 18: 36. 877-891.
36. Fuchs JR, Kavian A, Oh JT (2004) Diaphragmatic reconstruction with autologous tendon engineered from mesenchymal amniocytes. Journal of Pediatric Surgery 39: 834-838.
37. Gang EJ, Bosnakovski D, Figueiredo CA (2007) SSEAe4 identifies mesenchymal stem cells from bone marrow. Blood 109: 1743-1751.
38. Gao J, Coggeshall RE, Chung JM (2007) Functional motoneurons develop from human neural stem cell transplants in adult rats. Neuroreport 18: 565-569.
39. Gekas J, Walther G, Skuk D, Bujold E, Harvey I, et al. (2010) In vitro and in vivo study of human amniotic fluid-derived stem cell differentiation into myogenic lineage. Clin Exp Med 10: 1-6.
40. Ghionzoli M, Cananzi M, Zani A, Rossi CA, Leon FF, et al. (2010) Amniotic fluid stem cell migration after intraperitoneal injection in pup rats: implication for therapy. Pediatr Surg Int 26: 79-84.
41. Gidekel S, Pizov G, Bergman Y, Pikarsky E (2003) Oct-3/4 is a dose-dependent oncogenic fate determinant. Cancer Cell 4: 361-370.
42. Giuliani S, Perin L, Sedrakyan S, Kokorowski P, Jin D, et al. (2008) Ex vivo whole embryonic kidney culture: a novel method for research in development, regeneration and transplantation. J Urol 179: 365-370.
43. Gosden CM (1983) AF cell types and culture. British Medical Bulletin 39: 348-354.
44. Gosden C, Brock DJ (1978) Combined use of alphafetoprotein and amniotic fluid cell morphology in early prenatal diagnosis of fetal abnormalities. J Med Genet 15: 262-270.
45. Grisafi D, Piccoli M, Pozzobon M, Ditadi A, Zaramella P, et al. (2008) High transduction efficiency of human amniotic fluid stem cells mediated by adenovirus vectors. Stem Cells and Development 17: 953-962.
46. Gupta S, Verfaillie C, Chmielewski D, Kren S, Eidman K, et al. (2006) Isolation and characterization of kidney-derived stem cells. J Am Soc Nephrol 17: 3028-3040.
47. Gupta S, Verfaillie C, Chmielewski D, Kim Y, Rosenberg ME (2002) A role for extrarenal cells in the regeneration following acute renal failure. Kidney Int 62: 1285-1290.
48. Hoehn H, Bryant EM, Karp LE, Martin GM (1975) Cultivated cells from diagnostic amniocentesis in second trimester pregnancies. II. Cytogenetic parameters as functions of clonal type and preparative technique. Clin Genet 7: 29-36.
49. Hoehn H, Salk D (1982) Morphological and biochemical heterogeneity of amniotic fluid cells in culture. Methods Cell Biol 26: 11-34.
50. Hotta R, Natarajan D, Thapar N (2009) Potential of cell therapy to treat pediatric motility disorders. Semin Pediatr Surg 18: 263-273.
51. Igura K, Zhang X, Takahashi K, Mitsuru A, Yamaguchi S, et al. (2004) Isolation and characterization of mesenchymal progenitor cells from chorionic villi of human placenta. Cytotherapy 6: 543-553.
52. In 't Anker PS, Scherjon SA, Kleijburg-van der Keur C, Noort WA, Claas FH, et al. (2003) Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation. Blood 102: 1548-1549.
53. In 't Anker PS1, Scherjon SA, Kleijburg-van der Keur C, de Groot-Swings GM, Claas FH, et al. (2004) Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. Stem Cells 22: 1338-1345.
54. Iop L, Chiavegato A, Callegari A, Bollini S, Piccoli M, et al. (2008) Different cardiovascular potential of adult- and fetal-type mesenchymal stem cells in a rat model of heart cryoinjury. Cell Transplant 17: 679-694.
55. Itskovitz-Eldor J, Schuldiner M, Karsenti D, Eden A, Yanuka O, et al. (2000) Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. Mol Med 6: 88-95.

56. Karlmark KR, Freilinger A, Marton E, Rosner M, Lubec G, et al. (2005) Activation of ectopic Oct-4 and Rex-1 promoters in human amniotic fluid cells. Int J Mol Med 16: 987-992.
57. Kaviani A, Perry TE, Dzakovic A, Jennings RW, Ziegler MM, et al. (2001) The amniotic fluid as a source of cells for fetal tissue engineering. J Pediatr Surg 36: 1662-1665.
58. Kern S, Eichler H, Stoeve J, Klüter H, Bieback K (2006) Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 24: 1294-1301.
59. Khalil PN, Weiler V, Nelson PJ, Khalil MN, Moosmann S et al. (2007) Nonmyeloablative stem cell therapy enhances microcirculation and tissue regeneration in murine inflammatory bowel disease. Gastroenterology 132: 944-954.
60. Kim CF, Jackson EL, Woolfenden AE, Lawrence S, Babar I, et al. (2005) Identification of bronchioalveolar stem cells in normal lung and lung cancer. Cell 121: 823-835.
61. Kim J, Kang HM, Kim H, Kim MR, Kwon HC, et al. (2007) Ex vivo characteristics of human amniotic membrane-derived stem cells. Cloning Stem Cells 9: 581-594.
62. Kim J, Lee Y, Kim H, Hwang KJ, Kwon HC, et al. (2007) Human amniotic fluid-derived stem cells have characteristics of multipotent stem cells. Cell Prolif 40: 75-90.
63. Kim PG, Daley GQ (2009) Application of induced pluripotent stem cells to hematologic disease. Cytotherapy 11: 980-989.
64. Koelling S, Miosge N (2009) Stem cell therapy for cartilage regeneration in osteoarthritis. Expert Opin Biol Ther 9: 1399-1405.
65. Koike M, Sakaki S, Amano Y, Kurosawa H (2007) Characterization of embryoid bodies of mouse embryonic stem cells formed under various culture conditions and estimation of differentiation status of such bodies. J Biosci Bioeng 104: 294-299.
66. Kolambkar YM, Peister A, Soker S, Atala A, Guldberg RE (2007) Chondrogenic differentiation of amniotic fluid-derived stem cells. J Mol Histol 38: 405-413.
67. Kramer J, Steinhoff J, Klinger M, Fricke L, Rohwedel J (2006) Cells differentiated from mouse embryonic stem cells via embryoid bodies express renal marker molecules. Differentiation 74: 91-104.
68. Kunisaki SM, Fuchs JR, Kaviani A, Oh JT, LaVan DA, et al. (2006) Diaphragmatic repair through fetal tissue engineering: a comparison between mesenchymal amniocyte- and myoblast-based constructs. J Pediatr Surg 41: 34-39.
69. Kunisaki SM, Freedman DA, Fauza DO (2006) Fetal tracheal reconstruction with cartilaginous grafts engineered from mesenchymal amniocytes. J Pediatr Surg 41: 675-682.
70. Kunisaki SM, Armant M, Kao GS, Stevenson K, Kim H, et al. (2007) Tissue engineering from human mesenchymal amniocytes: a prelude to clinical trials. J Pediatr Surg 42: 974-979.
71. Lengner CJ, Camargo FD, Hochedlinger K, Welstead GG, Zaidi S, et al. (2007) Oct4 expression is not required for mouse somatic stem cell self-renewal. Cell Stem Cell 1: 403-415.
72. Li C, Zhou J, Shi G, Ma Y, Yang Y, et al. (2009) Pluripotency can be rapidly and efficiently induced in human amniotic fluid-derived cells. Hum Mol Genet 18: 4340-4349.
73. Liedtke S, Enczmann J, Waclawczyk S, Wernet P, Kögler G (2007) Oct4 and its pseudogenes confuse stem cell research. Cell Stem Cell 1: 364-366.
74. Looijenga LH, Stoop H, de Leeuw HP, de Gouveia Brazao CA, Gillis AJ, et al. (2003) POU5F1 (OCT3/4) identifies cells with pluripotent potential in human germ cell tumors. Cancer Res 63: 2244-2250.
75. Lotgering FK, Wallenburg HC (1986) Mechanisms of production and clearance of amniotic fluid. Semin Perinatol 10: 94-102.
76. Marcus AJ, Woodbury D (2008) Fetal stem cells from extra-embryonic tissues: do not discard. J Cell Mol Med 12: 730-742.
77. Marcus AJ, Coyne TM, Rauch J, Woodbury D, Black IB (2008) Isolation, characterization, and differentiation of stem cells derived from the rat amniotic membrane. Differentiation 76: 130-144.
78. Mescher EJ, Platzker AC, Ballard PL, Kitterman JA, Clements JA, et al. (1975) Ontogeny of tracheal fluid, pulmonary surfactant, and plasma corticoids in the fetal lamb. J Appl Physiol 39: 1017-1021.
79. Miki T, Lehmann T, Cai H, Stolz DB, Strom SC (2005) Stem cell characteristics of amniotic epithelial cells. Stem Cells 23: 1549-1559.
80. Miki T, Strom SC (2006) Amnion-derived pluripotent/multipotent stem cells. Stem Cell Rev 2: 133-142.
81. Milunsky A (1979) Genetic Disorder of the Fetus. New York: Plenum Press: 75-84.
82. Morigi M, Introna M, Imberti B, Corna D, Abbate M, et al. (2008) Human bone marrow mesenchymal stem cells accelerate recovery of acute renal injury and prolong survival in mice. Stem Cells 26: 2075-2082.
83. Morigi M, Rota C, Montemurro T, Montelatici E, Lo Cicero V, et al. (2010) Life-sparing effect of human cord blood-mesenchymal stem cells in experimental acute kidney injury. Stem Cells 28: 513-522.
84. Morito T, Muneta T, Hara K, Ju YJ, Mochizuki T, et al. (2008) Synovial fluid-derived mesenchymal stem cells increase after intra-articular ligament injury in humans. Rheumatology (Oxford) 47: 1137-1143.
85. Muller F, Dommergues M, Ville Y, Lewin F, Delvalez-Morichon N, et al. (1994) Amniotic fluid digestive enzymes: diagnostic value in fetal gastrointestinal obstructions. Prenat Diagn 14: 973-979.
86. Murray P, Camussi G, Davies JA, Edgar D, Hengstschlager M, et al. (2007) The KIDSTEM European Research Training Network: developing a stem cell based therapy to replace nephrons lost through reflux nephropathy. Organogenesis 3: 2-5.
87. Muscolo DL, Ayerza MA, Farfalli G, Aponte-Tinao LA (2010) Proximal tibia osteoarticular allografts in tumor limb salvage surgery. Clin Orthop Relat Res 468: 1396-1404.
88. Nadri S, Soleimani M (2007) Comparative analysis of mesenchymal stromal cells from murine bone marrow and amniotic fluid. Cytotherapy 9: 729-737.
89. Nelson MM (1973) Amniotic fluid cell culture and chromosome studies. In A. E. H. Emery (Ed.), Antenatal Diagnosis of Genetic Disease: 69-8.
90. Nichols J, Zevnik B, Anastassiadis K, Niwa H, Klewe-Nebenius D, et al. (1998) Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. Cell 95: 379-391.
91. Niwa H, Miyazaki J, Smith AG (2000) Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nat Genet 24: 372-376.
92. Ott HC, Matthiesen TS, Goh SK, Black LD, Kren SM, et al. (2008) Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. Nat Med 14: 213-221.
93. Owen M, Friedenstein AJ (1988) Stromal stem cells: marrow-derived osteogenic precursors. Ciba Found Symp 136: 42-60.
94. Pan HC, Yang DY, Chiu YT, Lai SZ, Wang YC, et al. (2006) Enhanced regeneration in injured sciatic nerve by human amniotic mesenchymal stem cell. J Clin Neurosci 13: 570-575.
95. Pan HC, Cheng FC, Chen CJ, Lai SZ, Lee CW, et al. (2007) Post-injury regeneration in rat sciatic nerve facilitated by neurotrophic factors secreted by amniotic fluid mesenchymal stem cells. J Clin Neurosci 14: 1089-1098.
96. Panés J, Salas A (2009) Mechanisms underlying the beneficial effects of stem cell therapies for inflammatory bowel diseases. Gut 58: 898-900.
97. Parolini O, Alviano F, Bagnara GP, Bilic G, Bühring HJ, et al. (2008) Concise review: isolation and characterization of cells from human term placenta: outcome of the first international Workshop on Placenta Derived Stem Cells. Stem Cells 26: 300-311.
98. Peister A, Deutsch ER, Kolambkar Y, Hutmacher DW, Guldberg RE (2009) Amniotic fluid stem cells produce robust mineral deposits on biodegradable scaffolds. Tissue Eng Part A 15: 3129-3138.
99. Perin L, Giuliani S, Jin D, Sedrakyan S, Carraro G, et al. (2007) Renal differentiation of amniotic fluid stem cells. Cell Prolif 40: 936-948.
100. Pesce M, Schöler HR (2001) Oct4: gatekeeper in the beginnings of mammalian development. Stem Cells, 19: 271-278.

101. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, et al. (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284: 143-147.
102. Porada CD, Zanjani ED, Almeida-Porad G (2006) Adult mesenchymal stem cells: a pluripotent population with multiple applications. Curr Stem Cell Res Ther 1: 365-369.
103. Price FD, Kuroda K, Rudnicki MA (2007) Stem cell based therapies to treat muscular dystrophy. Biochimica and Biophysica Acta 1772: 272-283.
104. Prusa AR, Marton E, Rosner M, Bernaschek G, Hengstschläger M (2003) Oct4-expressing cells in human AF: a new source for stem cell research? Hum Reprod 18: 1489-1493.
105. Rehni AK, Singh N, Jaggi AS, Singh M (2007) AF derived stem cells ameliorate focal cerebral ischaemia-reper-fusion injury induced behavioural deficits in mice. 183: 95-100.
106. Riboldi M, Simon C (2009) Extraembryonic tissues as a source of stem cells. Gynecol Endocrinol 25: 351-355.
107. Roubelakis MG1, Pappa KI, Bitsika V, Zagoura D, Vlahou A, et al. (2007) Molecular and proteomic characterization of human mesenchymal stem cells derived from amniotic fluid: comparison to bone marrow mesenchymal stem cells. Stem Cells Dev 16: 931-952.
108. Sakaguchi Y, Sekiya I, Yagishita K, Ichinose S, Shinomiya K, et al. (2004) Suspended cells from trabecular bone by collagenase digestion become virtually identical to mesenchymal stem cells obtained from marrow aspirates. Blood 104: 2728-2735.
109. Salgado AJ, Oliveira JT, Pedro AJ, Reis RL (2006) Adult stem cells in bone and cartilage tissue engineering. Curr Stem Cell Res Ther 1: 345-364.
110. Santos CC, Furth ME, Snyder EY, De Coppi P, Soker S, et al. (2008) Response to Do amniotic fluid-derived stem cells differentiate into neurons in vitro? Nature Biotechnology 26: 270-271.
111. Schöler HR, Balling R, Hatzopoulos AK, Suzuki N, Gruss P (1989) Octamer binding proteins confer transcriptional activity in early mouse embryogenesis. EMBO J 8: 2551-2557.
112. Seaberg RM, Smukler SR, Kieffer TJ, Enikolopov G, Asghar Z, et al. (2004) Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages. Nat Biotechnol 22: 1115-1124.
113. Sessarego N, Parodi A, Podestà M, Benvenuto F, Mogni M, et al. (2008) Multipotent mesenchymal stromal cells from amniotic fluid: solid perspectives for clinical application. Haematologica 93: 339-346.
114. 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.
115. Siegel N, Valli A, Fuchs C, Rosner M, Hengstschläger M (2009) Expression of mTOR pathway proteins in human amniotic fluid stem cells. Int J Mol Med 23: 779-784.
116. Siegel N, Valli A, Fuchs C, Rosner M, Hengstschläger M (2009). Induction of mesenchymal/epithelial marker expression in human amniotic fluid stem cells. Reprod Biomed On 19: 838-846.
117. Soncini M, Vertua E, Gibelli L, Zorzi F, Denegri M, et al. (2007) Isolation and characterization of mesenchymal cells from human fetal membranes. J Tissue Eng Regen Med 1: 296-305.
118. Stefanidis K, Loutradis D, Koumbi L, Anastasiadou V, Dinopoulou V, et al. (2008) Deleted in Azoospermia-Like (DAZL) gene-expressing cells in human amniotic fluid: a new source for germ cells research? Fertil Steril 90: 798-804.
119. Steigman SA, Ahmed A, Shanti RM, Tuan RS, Valim C, et al. (2009) Sternal repair with bone grafts engineered from amniotic mesenchymal stem cells. J Pediatr Surg 44: 1120-1126.
120. Streubel B, Martucci-Ivessa G, Fleck T, Bittner RE (1996) [In vitro transformation of amniotic cells to muscle cells--background and outlook]. Wien Med Wochenschr 146: 216-217.
121. Srivastava AS, Feng Z, Mishra R, Malhotra R, Kim HS, et al. (2007) Embryonic stem cells ameliorate piroxicam-induced colitis in IL10-/- KO mice. Biochem Biophys Res Commun 361: 953-959.
122. Tamagawa T, Ishiwata I, Saito S (2004) Establishment and characterization of a pluripotent stem cell line derived from human amniotic membranes and initiation of germ layers in vitro. Hum Cell 17: 125-130.
123. Thakar N, Priest RE, Priest JH (1982) Estrogen production by cultured amniotic fluid cells. Clinical Research, 30: 888A.
124. Torricelli F, Brizzi L, Bernabei PA, Gheri G, Di Lollo S, et al. (1993) Identification of hematopoietic progenitor cells in human amniotic fluid before the 12th week of gestation. Ital J Anat Embryol 98: 119-126.
125. Toselli M, Cerbai E, Rossi F, Cattaneo E (2008) Do amniotic fluid-derived stem cells differentiate into neurons in vitro? Nat Biotechnol 26: 269-270.
126. Tsai MS, Lee JL, Chang YJ, Hwang SM (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.
127. Tsai MS, Hwang SM, Tsai YL, Cheng FC, Lee JL, et al. (2006) Clonal amniotic fluid-derived stem cells express characteristics of both mesenchymal and neural stem cells. Biol Reprod 74: 545-551.
128. Tsai MS, Hwang SM, Chen KD, Lee YS, Hsu LW, et al. (2007) Functional network analysis of the transcriptomes of mesenchymal stem cells derived from amniotic fluid, amniotic membrane, cord blood, and bone marrow. Stem Cells 25: 2511-2523.
129. Uccelli A, Pistoia V, Moretta L (2007) Mesenchymal stem cells: a new strategy for immunosuppression? Trends Immunol 28: 219-226.
130. Underwood MA, Gilbert WM, Sherman MP (2005) Amniotic fluid: not just fetal urine anymore. J Perinatol 25: 341-348.
131. Ungrin MD, Joshi C, Nica A, Bauwens C, Zandstra PW (2008) Reproducible, ultra high-throughput formation of multicellular organization from single cell suspension-derived human embryonic stem cell aggregates. PLoS One 3: e1565.
132. Valli A, Rosner M, Fuchs C, Siegel N, Bishop CE, et al. (2010) Embryoid body formation of human amniotic fluid stem cells depends on mTOR. Oncogene 29: 966-977.
133. von Koskull H, Virtanen I, Lehto VP, Vartio T, Dahl D, et al. (1981) Glial and neuronal cells in amniotic fluid of anencephalic pregnancies. Prenat Diagn 1: 259-267.
134. von Koskull H, Aula P, Trejdosiewicz LK, Virtanen I (1984) Identification of cells from fetal bladder epithelium in human amniotic fluid. Hum Genet 65: 262-267.
135. Walther G, Gekas J, Bertrand OF (2009) Amniotic stem cells for cellular cardiomyoplasty: promises and premises. Catheter Cardiovasc Interv 73: 917-924.
136. Weiss DJ (2008) Stem cells and cell therapies for cystic fibrosis and other lung diseases. Pulm Pharmacol Ther 21: 588-594.
137. Wolbank S, Peterbauer A, Fahrner M, Hennerbichler S, van Griensven M, et al. (2007) Dose-dependent immunomodulatory effect of human stem cells from amniotic membrane: a comparison with human mesenchymal stem cells from adipose tissue. Tissue Eng 13: 1173-1183.
138. Yao M, Dieterle T, Hale SL, Dow JS, Kedes LH, et al. (2003) Long-term outcome of fetal cell transplantation on postinfarction ventricular remodeling and function. J Mol Cell Cardiol 35: 661-670.
139. Yeh YC, Wei HJ, Lee WY, Yu CL, Chang Y, et al. (2010) Cellular cardiomyoplasty with human amniotic fluid stem cells: in vitro and in vivo studies. Tissue Eng Part A 16: 1925-1936.
140. You Q, Tong X, Guan Y, Zhang D, Huang M, et al. (2009) The biological characteristics of human third trimester amniotic fluid stem cells. J Int Med Res 37: 105-112.
141. Zani A, Cananzi M, Eaton S, Pierro A, De Coppi P (2009) Stem cells as a potential treatment of necrotizing enterocolitis. J Pediatr Surg 44: 659-660.
142. Zhang P, Baxter J, Vinod K, Tulenko TN, Di Muzio PJ (2009) Endothelial differentiation of amniotic fluid-derived stem cells: synergism of biochemical and shear force stimuli. Stem Cells Dev 18: 1299-1308.

143. Zhao P, Ise H, Hongo M, Ota M, Konishi I, et al. (2005) Human amniotic mesenchymal cells have some characteristics of cardiomyocytes. Transplantation 79: 528-535.

144. Zsebo KM, Williams DA, Geissler EN, Broudy VC, Martin FH, et al. (1990) Stem cell factor is encoded at the Sl locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. Cell 63: 213-224.