Fetal stem cells from extra-embryonic tissues: do not discard

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Abstract

Stem cells hold promise to treat diseases currently unapproachable, including Parkinson’s disease, liver disease and diabetes. Seminal research has demonstrated the ability of embryonic and adult stem cells to differentiate into clinically useful cell types in vitro and in vivo. More recently, the potential of fetal stem cells derived from extra-embryonic tissues has been investigated. Fetal stem cells are particularly appealing for clinical applications. The cells are readily isolated from tissues normally discarded at birth, avoiding ethical concerns that plague the isolation embryonic stem cells. Extra-embryonic tissues are large, potentially increasing the number of stem cells that can be extracted. Lastly, the generation and sequestration of cells that form extra-embryonic tissues occurs early in development and may endow resident stem cell populations with enhanced potency. In this review we summarize recent work examining the plasticity and clinical potential of fetal stem cells isolated from extra-embryonic tissues.

Keywords: wharton’s jelly • amnion • amniotic fluid • multipotent • cell replacement

Stem cells – an abridged history

Stem cells can be loosely classified into three broad categories based on their time of isolation during ontogenesis: embryonic, fetal and adult. In this section they will be discussed not in order of their appearance in the organism, but arranged chronologically by date of initial isolation and characterization.

The first stem cell population was identified in adult mouse bone marrow by McCulloch and Till in the early 1960s. Pioneering work on these colony forming unit-spleen cells, later termed hematopoietic stem cells (HSCs), established the two functional properties of a stem cell population: self-renewal and multipotency. HSCs divide readily in culture and give rise to progeny that retain the colony-forming properties of the parental cells, evidence of self-renewal. Progeny of HSCs differentiate into multiple specialized cell types, including erythrocytes granulocytes and megakaryocytes, fulfilling the criteria of multipotency [1–3]. These landmark studies have formed the foundation of stem cell biology – revolutionizing the manner in which physicians and scientists study and treat disease.

Recent years have witnessed an explosion in the number of adult stem cell populations isolated and characterized. Every tissue or organ, from brain to fat, apparently contains a stem cell population. While
still multipotent, adult stem cells have long been considered restricted, giving rise only to progeny of their resident tissues. Recent, and currently controversial studies have challenged this dogma, suggesting that adult stem cells may be far more plastic than previously appreciated [4, 5]. If confirmed, these findings would have far reaching implications. Stem cells from easily harvested tissues (skin, bone marrow, fat) might be used clinically to treat disorders of more vulnerable and less accessible internal organs. Current research is investigating the utility of adult stem cells in the treatment of many disorders, including Parkinson’s disease, cardiovascular disease and diabetes.

The roots of embryonic stem cell (ESC) research can be traced back more than half a century. Examination of mouse teratomas, complex tumors containing a mix of differentiated adult tissues, hinted at the existence of ESCs [6–8]. However, the generation and characterization of ESC lines from the blastocysts of mice and humans did not occur until the early 1980s and late 1990s, respectively [9,10]. Subsequent studies have shown ESCs to fulfill the criteria of a stem cell population as first proposed by McCulloch and Till. ESCs divide indefinitely in culture (self-renewal) while maintaining their capacity for extensive in vitro and in vivo differentiation (pluripotency). Isolation from the earliest stages of development has endowed ESCs with the plasticity to differentiate to derivatives of all germ layers, including clinically important cell types such as dopaminergic neurons and pancreatic beta-cells [11, 12]. ESCs reintroduced into blastocysts can participate in the development of all organs and tissues in the adult animal, confirming their intrinsic potency [13]. This demonstrated plasticity has made ESCs the benchmark against which stem cell potency is measured. Despite ethical and political concerns, ESCs remain a leading candidate for future regenerative medicine applications.

Fetal stem cells

Extra-embryonic tissues as stem cell reservoirs offer many advantages over both embryonic and adult stem cell sources. Extra-embryonic tissues, collectively known as the afterbirth, are routinely discarded at parturition, so little ethical controversy attends the harvest of the resident stem cell populations. The extracorporeal nature of fetal stem cell sources facilitates isolation, eliminating patient risk that attends adult stem cell isolation. Most significantly, the comparatively large volume of extra-embryonic tissues and ease of physical manipulation theoretically increases the number of stem cells that can be isolated.

Cord blood represents the prototypical fetal stem cell source. HSCs isolated from cord blood have been extensively studied and have demonstrated clinical utility. Excellent reviews of cord blood stem cells have been recently published, and they will not be discussed in detail in this review [17, 18]. Study of other fetal stem cells lags behind, and much work will be required to see if these stem cell populations measure up to the high standard set by cord blood HSCs. What we do know about fetal stem cells from other extra-embryonic sources is summarized below.

Amniotic fluid (AF)

For more than 70 years AF has been used as a safe and reliable tool for prenatal diagnosis of genetic
disease. Recent evidence has suggested that AF may have utility beyond diagnostics, serving as an accessible reservoir of multipotent fetal cells. Of all the extra-ESC sources discussed in this review, AF is the only one where harvest typically occurs prior to parturition. This unique trait may become increasingly important as in utero cell-based therapies progress [19].

AF contains a heterogeneous population of cells displaying a range of morphologies. Most of these cells are epithelial in nature and have a limited capacity to proliferate in culture. Cells originating from the fetal skin, urogenital, respiratory and digestive tracts can be found within the AF. Additional cells from the inner surface of the amniotic membrane add to the mix. The cellular composition of AF changes with gestation, coinciding with the maturation of the fetus [20]. Inappropriate cell types, such as neural cells, can sometimes populate AF in cases of fetal anomalies [21]. The multitude of cell types existing within the AF lead to the hypothesis that stem cells might also be present. In fact, stem cells within AF were first isolated and described in 1993 by Toricelli et al. Cells with the characteristics of HSCs were isolated from human AF at 7–12 weeks of gestation [20]. This finding inspired further efforts to isolate additional stem cell populations from the AF.

The presence of mesenchymal cells in AF had been suggested for a number of decades, but the existence of a mesenchymal, non-hematopoietic, stem cell population has only recently been reported [22]. Amniotic fluid mesenchymal stem cells (AF-MSCs) were first isolated and characterized in 2003. AF-MSCs were initially isolated from human amniocentesis samples based on their preferential adherence to tissue culture plastic. These cells expressed a number of mesenchymal cell surface markers, including CD90 and CD105. Following in vitro expansion, the isolated cells were capable of differentiating in vitro into fibroblasts, adipocytes and osteoblasts. AF-MSCs can be isolated from as little as 2 ml of extracted AF from second trimester pregnancies, and expanded to >180 million cells within 4 weeks. This proliferative capacity meets or exceeds that described for adult human MSCs, suggesting that AF-MSCs may be particularly well suited for procedures requiring large numbers of donor cells [23].

Subsequent works have further characterized putative stem cell populations isolated from AF. Prusa et al. demonstrated the expression of Oct4 within a subset of AF cells. This is important, as Oct4 expression is associated with pluripotent cells such as embryonic germ cells and ES cells [24]. Demonstration of proliferation within this population further suggests that pluripotent stem cells can be both isolated and propagated from the AF of humans. Other groups have provided evidence that AF-MSCs express both mesodermal and ectodermal gene products [25, 26]. This is consistent with the emerging concept that stem cell populations exist in a multidifferentiated state. In the most comprehensive
study to date, De Coppi et al. have examined the potential of amniotic fluid-derived stem cells (AFS cells) isolated from rodents and humans. Employing immunoselection, AF cells expressing the cell surface antigen c-Kit were purified from primary amniocentesis cultures. Isolated cells grew rapidly in culture and were capable of more than 250 population doublings. This demonstrated proliferative capacity far exceeds the Hayflick limit of 50 doublings established for most cultured somatic cells. Importantly, AFS cells display a normal karyotype and maintain telomere length during long-term culture. This latter attribute facilitated the establishment of clonal lines from AFS cells, necessary to establish ‘stemness’ of a population. Clonal AFS lines differentiated in vitro to putative adipocytes, endothelial cells, hepatocytes, osteocytes, myocytes and neurons, derivatives of all germ layers (Fig. 2). This broad plasticity appeared to be a general attribute of the selected cells: nineteen different amniocentesis cultures yielded multipotent AFS cell clonal lines [27].

Most relevant to ultimate clinical application, AF cells have been transplanted into animal models of disease. AF cells injected into the ischemic myocardium did not differentiate to cardiomyocytes, but did participate in neovascularization [30]. However, a more recent study documented rapid and complete rejection of AFS cells transplanted to a similar ischemic model [31]. More work is needed to resolve these apparently contradictory outcomes.

Survival of transplanted AF cells appears more robust in the brain. AF cells transplanted to the normal or ischemic brain survive and differentiate to neural lineages, predominantly astrocytes [32]. Clonal lines of human AFS cells pre-differentiated into neural cells in vitro engraft into a number of brain areas following transplantation to the mouse brain. Interestingly, the same clonal line was capable of bone formation under a different in vivo paradigm, further evidence of the plasticity and therapeutic potential of AFS cells. In all cases, the transplanted cells survived, appeared well behaved and did not show any evidence of tumor formation [27].

**Wharton’s jelly**

The umbilical cord contains two arteries and one vein, protected by a proteoglycan rich connective tissue called Wharton’s jelly. Within the abundant extracellular matrix of Wharton’s jelly resides a recently described stem cell population called Umbilical Cord Matrix Stem Cells (UCMSCs). The cells are present in relatively high numbers, with an average of 400,000 cells isolated per umbilical cord [33]. This is significantly greater than the number of...
MSCs that can be routinely isolated from adult bone marrow, emphasizing a primary advantage of stem cell harvest from extra-embryonic sources. The isolated cells expressed CD29 and CD54, consistent with a mesenchymal cell type, and could be propagated in culture for more than 80 population doublings. UCMSCs expressed several stem cell markers, including c-Kit, Nanog, Oct4 and Sox2 [34, 35]. In vitro, the cells were capable of differentiation to multiple mesodermal cell types, including fat, bone and skeletal muscle [36, 37]. More surprisingly, defined in vitro treatments favored differentiation to putative cardiomyocytes and neurons [34, 37]. Generation of clinically important dopaminergic neurons has also been reported [38].

In a more recent study, Karahuseyinoglu et al. have demonstrated the existence of at least two apparently distinct progenitor cell populations within the umbilical cord matrix. Initially distinguished based on morphology, these type 1 and type 2 cells can be further identified by their differential expression of vimentin and cytokeratins [33]. Both populations of cells are multipotent, capable of differentiation to fat, bone and cartilage. Comparison to prototypical adult bone marrow stromal cells (MSCs) has revealed interesting differences. Adult MSCs appear to be more adept at in vitro differentiation to adipocytes, demonstrating more rapid lipid accumulation and attaining morphologic maturity more readily than UCMSCs. In contrast, UCMSCs were far more capable of chondrogenic differentiation than adult MSCs (Fig. 3). Grown in pellet cultures with chondrogenic media, the UCMSCs formed tightly compacted spheres with a smooth outer surface. The spheres stained for mucopolysaccharides and collagen II, consistent with chondrogenic differentiation. Staining was more intense than that demonstrated for adult MSCs grown under identical conditions. These findings suggest that UCMSCs may be prime candidates for cartilage repair in future clinical applications.

**Fig. 3** Chondrogenically induced human umbilical cord stromal cells (HUCSCs) formed tiny cell spheres. (A): A shiny-surfaced cell mass. (B): Non-induced cells formed smaller, bulky cell masses. (C): Toluendine blue stain shows the mucopolysaccharide-rich extracellular matrix (pinkish metachromatic areas) and a clear capsule surrounding the entire sphere (arrowhead). (C'): No metachromasia was noted in irregular masses of non-induced cells. (D): In azan-stained cell masses, collagen fibers were clearly distinguished (arrowheads) among many chondrocytes (nuclei in pale red). (E): In cell masses built by the induction of bone marrow MSCs, cells appeared as small groups. Abundant type II (F) and few type I collagen fibers (G) (arrowheads) were detected in chondrogenically induced HUCSCs. (H): Only a few type II collagen fibers were noted in induced bone marrow MSCs. Scale bars = 50 µm (F, G), 100 µm (D, E), 200 µm (C'), and 500 µm (A–C). Reprinted with permission Stem Cells Vol. 25 No. 2 February 2007, pp. 319–31.
Intriguingly, type 1 and type 2 UCMSCs differed in their ability to generate non-mesodermal derivatives. Under defined in vitro conditions type 2 differentiated to putative neurons, while type 1 cells did not. Type 2 cells exposed to induction media extended processes and expressed β-III-tubulin, neurofilament-M (NF-M) and NeuN, consistent with early neuronal differentiation. In contrast, type 1 cells remained unaltered, with no significant morphological changes apparent. In these studies neuronal differentiation of type 2 cells was not followed by consolidation of phenotype, and the cells eventually reverted to a more fibroblastic state. Nevertheless, these results suggest divergent plasticity between umbilical cord matrix stem cell populations, and underscore the importance of further analysis and characterization.

In vivo studies involving the transplantation of UCMSCs are limited, but encouraging. UCMSCs transplanted in a mouse model of severe muscle damage survived for at least 2 weeks. The donor cells expressed proteins consistent with skeletal muscle differentiation and enhanced muscle regeneration [36]. Transplantation of UCMSCs to the brain has also been performed. UCMSCs placed in the ischemic rodent brain improved the neurological function of recipients as compared to non-transplant controls. Donor cells showed evidence of differentiation to neurons, glia and vascular endothelial cells, consistent with the multipotency displayed in vitro. Transplantation promoted blood vessel formation and increased local blood flow in the area of the ischemic lesion. Expression of the neurotrophic factors brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) was also enhanced by stem cell transplantation. These studies suggest that transplanted UCMSCs may provide clinical benefit through multiple pathways [39]. Other work has documented the ability of UCMSCs transplanted to the striatum of hemiparkinsonian rats to ameliorate apomorphine-induced rotations. Importantly, there was no evidence of tumor formation up to 12 weeks post-transplantation [35]. UCMSCs may therefore be useful for the treatment of a number of debilitative neurological disorders.

Placenta

The placenta is a large fetomaternal organ that provides nourishment to the developing fetus. The sheer volume of the placenta as compared to other extra-embryonic structures makes this tissue particularly attractive for the isolation of multipotent cells. For example, a full term human placenta on average weighs more than 590 g, almost 15 times the weight of the average umbilical cord [40]. Commercial ventures aimed at exploiting this vast potential reservoir have recently been announced (www.celgene.com).

Placental-derived stem cells (PDSCs) can be obtained from dissociated placental tissue based on plastic adherence, a technique widely employed for the isolation of bone marrow mesenchymal cells. Not surprisingly, PDSCs express numerous mesenchymal surface markers, including CD29 and CD44. In vitro, these cells can substitute for bone marrow-derived MSCs, supporting the growth of exogenous HSCs derived from cord blood [41]. PDSCs display fibroblastic morphologies and express the pluripotency markers Oct4 and Rex1. Similar to bone marrow MSCs, the placental-derived cells exist in a multidifferentiated state—simultaneously expressing ectodermal, mesodermal and endodermal genes. PDSCs are highly proliferative and can be maintained for at least 20 passages in culture. Under defined in vitro conditions, PDSCs differentiated to putative osteoblasts and adipocytes. Importantly, clonal lines established from single cells are capable of self-renewal and are multipotent, indicating that PDSCs represent a true stem cell population [42].

In vitro differentiation of PDSCs is not limited to osteoblasts and adipocytes. Differentiation to mesodermal chondrocyte-like, myocyte-like and ectodermal neuron-like derivatives has been reported [43]. More recently, differentiation to endodermal hepatocyte-like cells has been achieved in vitro [44]. In the latter study, PDSCs were isolated from human afterbirths, dissociated and cultured in serum containing media. Flow cytometry identified the isolated cells as CD90+/CD105+ and CD34−, consistent with a PDSC phenotype. Cells cultured in expansion media were uniformly negative for expression of the hepatocyte markers CK18 and albumin. Exposure to hepatocyte growth factor (HGF) and FGF4 up-regulated the expression of these markers within 1 week of culture, and expression was maintained for at least 4 weeks. Interestingly, differentiation was enhanced when the PDSCs were plated on poly-L-lysine coated dishes, suggesting that both soluble and insoluble factors play a role in hepatocyte differentiation.
Functional properties of mature hepatocytes were also present in the differentiated cells, including internalization of low-density lipoprotein (LDL) (Fig. 4). Control cultures of PDSCs not exposed to differentiation cues were incapable of performing this task. Similarly, glycogen storage was only detected in the differentiated cells.

Together, these results demonstrate PDSC differentiation to mesodermal, ectodermal and endodermal cell types, suggesting plasticity rivaling that of ES cells.

Clinical utility of stem cell populations often implies transplantation of individual cell, either undifferentiated or pre-differentiated in vitro. A complimentary approach is the ex vivo engineering of complex biological structures. PDSCs have been employed in the latter scenario to construct artificial heart valves. PDSCs seeded onto biodegradable scaffolds generated complex structures with mechanical properties similar to native heart valves [45]. Such bioengineering approaches are
not unique to PDSCs and could enhance the utility fetal stem cell populations in general. 

In vivo studies have shown that PDSCs stereotac-
tically implanted into the intact adult rat brain persist-
ed for up to 3 months, showed migratory activity and
assumed typical neuron-like morphologies. Similarly,
transplantation to tissues in the periphery resulted in
appropriate differentiation to mesodermal bone and
cartilage. In complimentary studies, PDSCs infused
into fetal sheep in utero resulted in widespread inte-
gration into multiple organ systems, including bone
marrow, liver and heart. These in vivo results are con-
sistent with the plasticity ascribed in vitro, and provide
further evidence of the potency of PDSCs [46].

Amniotic membrane

The amniotic membrane or amnion, delineates the
gestational sac, a highly resilient, transparent, fluid-
filled cavity that encompasses a developing fetus dur-
ing gestation. This structure is generated very early in
development and is one of the first recognizable tis-
sues derived from the epiblast. The amnion is an avas-
cular structure consisting of three discrete layers: an
inner epithelial layer, an interposing, acellular base-
ment membrane and an outer layer of mesodermal
cells [47]. This relatively simple structure belies the
complexity of its origins. In the human, amniotic
epithelial cells (AECs) differentiate from the epiblast at
the end of the first week of gestation to form the inner
layer of the amniotic membrane. In parallel, extra-
embryonic somatopleuric mesodermal cells, derived
from the caudal end of the epiblast (in the region of the
primitive streak), line the outer surface of the mem-
brane. Consequently, the amnion is unique: generated
early in development from multipotent cells residing in
two distinct areas of the developing blastocyst. During
subsequent development, the cells of the amnion are
not exposed to the same barrage of signals responsi-
ble for gradual fate restriction of cells within the
embryo proper. It is postulated that stem cell popula-
tions sequestered within the amnion might retain the
potency of the epiblast cells from which they arose.

Indeed, several multipotent cells have been isolat-
ed from the amnion, including AECs, amniotic mes-
enchymal cells (AMCs) and amnion-derived stem
cells (ADSCs). The populations share characteris-
tics, yet differ in many respects. These differences
may in turn endow each population with unique ther-
apeutic advantages. Similar to most fetal stem cells,
amnion-derived multipotent cells are thought to be
immunoprivileged. Intact amniotic membrane has a
long history of clinical utility. Amniotic membrane has
been extensively used as a biological dressing to
treat chemical and thermal burns. The clinical suc-
cess of amniotic membrane transplantation is due in
part to its immunoprivileged characteristics. If this
trait is indeed shared by its constituent stem cell pop-
ulations they may be particularly well suited for allo-
genic transplantation strategies.

The best-characterized multipotent cells isolated
from the amnion are the AECs, residents of the
innermost layer of the gestational sac. In the most
extensive study to date, Miki et al. isolated AECs
from human afterbirths obtained following cesearian
section [48]. In vitro, the AECs displayed epithelial
morphologies and grew into a tightly packed, cobble-
stone monolayer in culture. With time, clusters of
cells loosely attached to the adherent monolayer
became evident. These loosely adherent cell clusters
contained high proportions of putative stem cells.
Subsets of AECs were positive for the stem cell sur-
face antigens SSEA-3, SSEA-4, TRA-1-60 and TRA-
1-81. In addition, the cells expressed Sox2, Oct4,
nanog, Rex1 and FGF4, gene products associated
with pluripotent ES cells. Expression of telomerase
reverse transcriptase (TERT), another ES cell mark-
er, was not detected. This is consistent with the limit-
ed in vitro proliferation reported.

The expression of numerous stem cell markers
suggests that AECs may be multipotent; a contention
supported by in vitro differentiation studies (Fig. 5).
To assess capacity for endodermal differentiation,
AECs were grown in the presence of nicotinamide
for 7 days. Treated cells initiated the expression of
multiple pancreatic genes, including the transcription
factor Pax-6 and the hormones glucagon and insulin.
Different culture conditions encouraged hepatic dif-
ferentiation, as demonstrated by expression of albu-
min and α1-antitrypsin.

In vitro differentiation to mesodermal cardiomy-
cytes was also achieved. Exposure of AECs to
ascorbic acid 2-phosphate resulted in the robust
expression of the cardiac-specific genes atrial and
ventricular myosin light chain 2 (MLC-2A and MLC-
2V, respectively).

Lastly, AECs were capable of in vitro differen-
tiation to ectodermal neural cells. Freshly isolated
AECs constitutively express a number of neural genes, including neural-specific enolase (NSE), NF-M and myelin basic protein (MBP), perhaps suggesting a predilection for neural differentiation. Exposure to all-trans retinoic acid and FGF4 resulted in adoption of an elongated, neural morphology and enhanced expression of nestin and glutamic acid decarboxylase (GAD). Differentiation to astrocyte-like and oligodendrocyte-like cells was evidenced by expression of glial fibrillary acidic protein (GFAP) and cyclic nucleotide phosphodiesterase (CNP), respectively.

To summarize, the study by Miki et al. strongly suggests that AECs are highly plastic and undergo early stages of differentiation to derivatives of all germ layers. More study is needed to determine whether or not this differentiation can be consolidated, in vitro or in vivo, to render fully functional cells for clinical therapy.

As anticipated, AECs appear to have immunoprivileged characteristics: they do not express HLA-A, B, C and DR antigens, beta 2-microglobulin or MHC Class II antigens [49–51]. In addition, AECs secrete a number of immunosuppressive factors that target the innate and adaptive immune systems, which may support survival following transplantation [52, 53]. Consistent with these observations, donor AECs...
survive long-term following transplantation to animal models. AECs transplanted to the livers of syngeneic fetal and adult rats integrated and survived for at least 14 days and 30 days, respectively [54, 55]. The focus of these studies was the utility of AECs as vectors to carry therapeutic genes, therefore tissue specific differentiation was not analyzed.

AECs have been examined in particular for their utility in animal models of neurological disease. Encouraged by the inherent synthesis of dopamine by AECs, Kakishita et al. Transplanted cultured human cells into a rat model of Parkinson’s disease. Two weeks post-transplantation grafts demonstrated survival without uncontrolled growth. Tyrosine hydroxylase (TH)-immunoreactive cells were present within the grafts and partial amelioration of apomorphine-induced rotations was achieved [56]. In a follow-up study, increased survival of endogenous dopaminergic neurons in the midbrain was seen in transplanted animals, suggesting that transplantation of AECs may be a viable approach for the treatment of Parkinson’s disease [57]. Transplantation of AECs in animal models of lysosomal storage disease and spinal cord injury has also yielded encouraging results [58, 59].

Amnion mesenchymal cells (AMCs) have also been isolated from the amniotic membrane. These cells arise from the outer layer of the amnion, juxtaposed to the chorion. Similar to AECs, AMCs demonstrate low to no expression of HLA class I and HLA class II, suggesting they would be well tolerated following transplantation. In vitro, AMCs have some features of neuronal progenitor cells, expressing the neural stem cell markers nestin and Musashi1. In vitro differentiation to neuron-like cells may reflect a predisposition for neural differentiation [60]. Other studies have demonstrated that AMCs are capable of differentiation to cardiomyocyte-like cells and putative hepatocytes in vitro and/or in vivo [61].

A recent addition to the mix of multipotent cells derived from the amnion are the ADSCs. These cells were isolated from rat amnion using a tissue explant methodology that discourages the growth of AECs. Cells migrating from the amniotic membrane expressed the cell surface markers CD29 and CD90, suggesting that they originate from the outer somatopleuric layer. Cultured ADSCs express nanog, sox2 and TERT, genes associated with pluripotency and self-renewal. ADSCs can be greatly expanded in vitro and maintained for more than 50 passages. Gene expression analysis demonstrated that populations of ADSCs express a suite of ectodermal, mesodermal and endodermal genes prior to in vitro differentiation. When placed in appropriate in vitro conditions, ADSCs are capable of differentiation to osteoblast-, adipocyte-, hepatocyte- and neuron-like cells, derivatives of all three germ layers.

The proliferative capacity of ADSCs has allowed for the establishment of clonal lines derived from established ADSC populations. Clonal ADSC lines express the same combination of cell surface markers as the parental population, and demonstrate robust proliferation in culture. In vitro differentiation of clonal ADSC lines to endodermal, mesodermal and ectodermal derivatives has been achieved. Together these data show that ADSCs are true stem cells, and provide evidence that the amniotic membrane is a rich source of stem cell populations [62].

To date, ADSCs have only been isolated and characterized from rodent sources. Further research is needed to demonstrate that human homologues of ADSCs exist and can be adequately manipulated ex vivo to allow for therapeutic exploitation [62].

### Summary and conclusions

Stem cell-based regenerative medicine represents a novel approach to the treatment of disease, potentially revolutionizing medicine. A wide spectrum of disorders affecting diverse organ systems might be targeted through stem cell therapies. While the versatility displayed by adult and ESCs is encouraging, it seems unlikely that any single stem cell population will be ideal for all treatment scenarios. Therefore, a continued effort to identify and characterize novel stem cell populations appears critical for widespread clinical success. In recent years, fetal stem cells isolated from extra-embryonic tissues have been added to the growing list of putative stem cell populations, and may offer some advantages. Isolation from tissues normally discarded at birth facilitates harvest and overcomes ethical concerns. Fetal cells grow well in culture, appear capable of differentiation to multiple cell types and may be less likely to be rejected following transplantation. Additional study is required to fully assess the potential of fetal stem cell populations, however, they seem poised to join embryonic and adult stem cells as participants in the emerging field of regenerative medicine.
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