Biological characteristics of stem cells from foetal, cord blood and extraembryonic tissues

Hassan Abdulrazzak, Dafni Moschidou, Gemma Jones and Pascale V. Guillot*

Institute of Reproductive and Developmental Biology, Imperial College London, London W12 0NN, UK

Foetal stem cells (FSCs) can be isolated during gestation from many different tissues such as blood, liver and bone marrow as well as from a variety of extraembryonic tissues such as amniotic fluid and placenta. Strong evidence suggests that these cells differ on many biological aspects such as growth kinetics, morphology, immunophenotype, differentiation potential and engraftment capacity in vivo. Despite these differences, FSCs appear to be more primitive and have greater multi-potentiality than their adult counterparts. For example, foetal blood haemopoietic stem cells proliferate more rapidly than those found in cord blood or adult bone marrow. These features have led to FSCs being investigated for pre- and post-natal cell therapy and regenerative medicine applications. The cells have been used in pre-clinical studies to treat a wide range of diseases such as skeletal dysplasia, diaphragmatic hernia and respiratory failure, white matter damage, renal pathologies as well as cancers. Their intermediate state between adult and embryonic stem cells also makes them an ideal candidate for reprogramming to the pluripotent status.

Keywords: stem cell; foetal; regenerative medicine; induced pluripotent stem

1. INTRODUCTION

Stem cells are undifferentiated cells with the capacity to self-renew, differentiate and repopulate a host in vivo (Weissman et al. 2001). Their plasticity or potency is hierarchical ranging from totipotent (differentiating into all cell types including placenta), pluripotent (differentiating into cells of the three germ layers, ectoderm, mesoderm and endoderm, but not trophoblastic cells), multipotent (differentiating into cells of more than one type but not necessarily into all the cells of a given germ layer) to unipotent (differentiating into one type of cell only, e.g. muscle or neuron).

Pluripotent stem cells can be derived from the inner cell mass of the pre-implantation embryo (i.e. embryonic stem (ES) cells) or isolated from the foetal primordial germ cell pool (PGC) above the allantois (i.e. embryonic germ (EG) cells and embryonic carcinoma (EC) cells; Thomson et al. 1998; Andrews et al. 2005). The destruction of the blastocyst or early foetus necessary for their derivation/isolation raises ethical concerns (Lo & Parham 2009), although recent work has shown that ES cells can be derived from single blastomeres isolated using procedures similar to those routinely used for pre-implantation genetic diagnosis (Klimanskaya et al. 2007). However, safety concerns still remain because of the tumorigenicity of ES cells. Alternatively, adult stem cells can be found in almost all tissues examined including brain, dental pulp, muscle, bone marrow, skin and pancreas and have been extensively characterized for their therapeutic potential. The adult stem cell could be multipotent (e.g. haematopoietic stem cells (HSCs) giving rise to all blood cells and adherent stromal/mesenchymal stem cells (MSCs) that give rise to bone, fat, cartilage and muscle) or unipotent (e.g. progenitor cells). Adult MSCs have the problem of being difficult to extract in sufficient numbers for therapy and/or presenting restricted plasticity and limited proliferative capacity compared with ES cells.

In recent years, foetal stem cells (FSCs) and stem cells isolated from cord blood or extraembryonic tissues have emerged as a potential ‘half way house’ between ES cells and adult stem cells. FSCs can be found in foetal tissues such as blood, liver, bone marrow, pancreas, spleen and kidney, and stem cells are also found in cord blood and extraembryonic tissues such as amniotic fluid, placenta and amnion (Marcus &
Woodbury 2008). Their primitive properties, expansion potential and lack of tumorigenicity make them an attractive option for regenerative medicine in cell therapy and tissue engineering settings. While extraembryonic tissues could be used with few ethical reservations, the isolation of FSCs from abortuses is subject to significant public unease. We review here the phenotypic characteristics of stem cells from foetal, cord blood and extraembryonic tissues, their application in cell therapy and their potential for reprogramming towards pluripotentiality.

2. PHENOTYPIC CHARACTERISTICS OF FOETAL STEM CELLS

Stem cells are collected from abortal foetal tissue, surplus of pre-natal diagnostic tissues or tissues at delivery, subject to informed consent, institutional ethics approval and compliance with national guidelines covering foetal tissue research. Until recently they were thought to be multi-potent (O’Donoghue & Fisk 2004), but this picture is now changing as evidence is mounting regarding the existence of pluripotent sub-populations in some foetal and extraembryonic tissues (Cananzi et al. 2009). Some of the properties of the cells are summarized in table 1.

2.1. Foetal tissues

2.1.1. Foetal MSCs. MSCs are multi-potent stem cells that can differentiate towards mesoderm-derived lineages (i.e. osteogenic, adipogenic, chondrogenic and myogenic; Pittenger et al. 1999). Their potential to repair damaged tissue and their immunomodulatory properties make them very attractive for a wide range of regenerative medicine applications such as cell therapy and tissue engineering for hollow and solid organs (Horwitz et al. 2002; Abdallah & Kassem 2008; Le Blanc et al. 2008). They were first identified in adult bone marrow where they represent 0.001–0.01% of total nucleated cells (Owen & Friedenstein 1988). Adherent stromal cells with similar characteristics were subsequently isolated from other tissues such as adipose, dental pulp, muscle, liver and brain (Porada et al. 2006; Huang et al. 2009). They are also found in foetal and extraembryonic tissues, where they seem to possess greater proliferation capacity and differentiation potential than their adult counterparts (Campagnoli et al. 2001; Guillot et al. 2007b; Pappa & Anagnou 2009).

2.1.1.1. Common characteristics. MSCs isolated from foetal tissues such as blood, liver, bone marrow, lung and pancreas all share common characteristics. For example, they are spindle-shaped cells with the capacity to differentiate into the standard mesenchymal lineages, i.e. bone, fat and cartilage. They do not express haematopoietic or endothelial markers (e.g. they are CD45−/34−/14− and von Willebrand factor negative; Gucciardo et al. 2009), but expressstroma-associated markers CD29 (B1-integrin), CD73 (SH3 and SH4), CD105 (SH2), CD44 (HCAM1), the early bone marrow progenitor cell marker CD90 (thy-1) and the extracellular matrix proteins vimentin, laminin and fibronectin (Guillot et al. 2006, 2007a). Contrary to adult bone marrow MSCs, first-trimester foetal blood, liver and bone marrow MSCs express baseline levels of the pluripotency stem cell markers Oct-4, Nanog, Rex-1, SSEA-3, SSEA-4, Tra-1-60 and Tra-1-81 (Guillot et al. 2007b; Zhang et al. 2009). Regardless of their tissue of origin, first-trimester foetal MSCs self-renew faster in culture than adult MSCs (30–35 h versus 80–100 h) and senesce later while retaining a stable phenotype (Guillot et al. 2007b). Foetal MSCs are therefore more readily expandable to therapeutic scales for either pre- or post-natal ex vivo gene or cell therapy and tissue engineering. Having greater multi-potentiality and differentiating more readily than adult MSCs into cells of mesodermal origin such as bone and muscle, they can also differentiate into cells from other lineages such as oligodendrocytes and haematopoietic cells (MacKenzie et al. 2001; Chan et al. 2006; Kennea et al. 2009). In terms of engraftment, they have a competitive advantage compared with adult cells (Rebel et al. 1996; Harrison et al. 1997; Taylor et al. 2002). Other advantageous characteristics relevant to cell therapy include having active telomerase (Guillot et al. 2007b), expressing low levels of HLA I and lacking intracellular HLA II, and taking longer to express this upon interferon γ stimulation than adult MSCs (Gotherstrom et al. 2004). Foetal MSCs also express a shared α2, α4 and α5β1 integrin profile with first-trimester HSCs, implicating them in homing and engraftment, and, consistent with this, they have significantly greater binding to their respective extracellular matrix ligands than adult MSCs (de la Fuente et al. 2003). Finally, human foetal MSCs are readily transducible with transduction efficiencies of greater than 95 per cent using lentiviral vectors with stable gene expression at both short- and long-time points without affecting self-renewal or multi-potentiality (Chan et al. 2005).

2.1.1.2. Specific characteristics. Although the above characteristics are shared among the various types of foetal stem, they also have specific traits that are dependent on tissue of origin and gestational age. For example, foetal MSCs are present in foetal blood from the earliest gestation tested, seven weeks, where they account for approximately 0.4 per cent of foetal nucleated cells. Their numbers decline to very low levels after 13 weeks’ gestation (Campagnoli et al. 2001). MSC derived from foetal bone marrow also decline with age with one MSC found among 10 000 in mid-trimester as compared with one MSC per 250 000 cells in adult marrow.

Significant variations have been found among foetal tissue cells in terms of cell surface markers. For example, foetal lung has a higher proportion of CD34+/CD45− cells (44%) than bone marrow (4.8%), spleen (12.6%) and liver (7.5%) (in’t Anker et al. 2003a,b). Also foetal metanephric MSCs do not express CD45, CD34 or other haematopoietic markers; instead, they express high levels of mesenchymal markers such as vimentin, laminin and type I collagen.

There is also variation among the cells in terms of differentiation capacity. MSCs derived from foetal liver during the first and second trimester have a reduced osteogenic differentiation potential in
Table 1. Characteristics of stem cells. E, embryonic; MSC, mesenchymal; H, hematopoietic; EP, epithelial; VSEL, very small embryonic-like; P, pluripotent; M, multipotent; U, unipotent; n.d., not determined. Asterisk denotes that the characteristics of these cells are not listed in the table (Kyo et al. 1997; Campagnoli et al. 2001; Gammaitoni et al. 2004; Miki et al. 2005, 2007; Pranke et al. 2005; Dan et al. 2006; Habich et al. 2006; Miki & Strom 2006; Portmann-Lanz et al. 2006; Zhao et al. 2006; De Coppi et al. 2007; Fong et al. 2007; Guillot et al. 2007; Ilancheran et al. 2007; Karahuseyinoglu et al. 2007; Kim et al. 2007; Li et al. 2007; Musina et al. 2007; Prat-Vidal et al. 2007; Degistirici et al. 2008; Moon et al. 2008; Mountford 2008; Poloni et al. 2008; Sessarego et al. 2008; Troyer & Weiss 2008; Brooke et al. 2009; Cananzi et al. 2009; Riekstina et al. 2009; Zhang et al. 2009; Alaminos et al. 2010; Anzalone et al. 2010; Castrechini et al. 2010; Hsieh et al. 2010; Insausti et al. 2010; Vaziri et al. 2010).

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Comparison with first-trimester foetal blood and with second-trimester spleen, lung and bone marrow MSCs, possibly because of a reduction in osteogenic progenitor numbers (in’t Anker et al. 2003a,b). Foetal pancreatic MSCs, shown to be capable of differentiating into chondrogenic, osteogenic or adipogenic lineages (Hu et al. 2003), can also engraft, differentiate and secrete human insulin in a sheep model (Ersek et al. 2010). Moreover, foetal pancreatic ductal stem cells can differentiate into insulin-producing cells in vitro (Yao et al. 2004).

Foetal metanephric MSCs can be induced in vitro to adopt an osteogenic or myogenic phenotype and, when transplanted in a foetal lamb model, they show long-term persistence and site-specific differentiation (Almeida-Porada et al. 2002).

Altogether these data indicate a high level of heterogeneity within the foetal stem cell pool.

2.1.2. Foetal HSCs. HSCs are multi-potent stem cells that maintain functional haematopoiesis by generation of all haematopoietic lineages throughout foetal and adult life (Weissman & Shizuru 2008). They are characterized by the expression of CD34 and CD45 antigens, and the absence of markers such as CD38 and human leucocyte antigen (HLA)/DRE (Huss 2000; Taylor et al. 2002). During ontogeny the site of haematopoiesis is modified several times (Cumano et al. 2001). An area along the dorsal embryonic aorta termed the aorta-gonad-mesonephros (AGM) is a rich source of HSCs, which then migrate to the embryonic liver and later seed other haematopoietic tissues such as the bone marrow (McGrath & Palis 2008). HSCs are usually assayed in animal models based on their capacity to repopulate the entire haematopoietic system in conditioned recipients after transplantation. Similar to MSCs, it is possible to transplant human cells into xenogeneic recipients such as foetal sheep that act as models of human haematopoiesis (O’Donoghue & Fisk 2004).

First-trimester foetal blood contains more CD34+ cells than term gestation blood, yet CD34+ cells account for only 5 per cent of CD45+ cells in the blood at that stage (Campagnoli et al. 2001). The number of circulating HSCs increases from the first to the second trimester in utero, probably because of cells migrating from the foetal liver to establish haematopoiesis in the foetal bone marrow (Clapp et al. 1995). Some HSCs remain in the umbilical cord at delivery, where they can be collected for allogeneic or occasionally autologous cell transplantation (see §2.2.1.1).

In the second trimester, CD34+ cells constitute 4 per cent of cells in blood, 16.5 per cent in bone marrow, 6 per cent in liver, 5 per cent in spleen and 1.1 per cent in the thymus (Lim et al. 2005). This frequency of CD34+ cells in the blood gradually diminishes during the third trimester, probably reflecting establishment of the marrow as the primary site of haematopoiesis and the declining role of the foetal liver in that regard (Jones et al. 1994; Wagers et al. 2002). The number of cells negative for CD38 within the CD34+ population is also higher in early foetal blood, suggesting that these cells are more primitive and have greater potential than HSCs circulating later in ontogeny (O’Donoghue & Fisk 2004). Even relatively differentiated erythroblasts are more primitive in first compared with later trimester foetal blood. Foetal blood HSCs proliferate more rapidly than those in cord blood or adult bone marrow, and produce all haematopoietic lineages (Campagnoli et al. 2001).

Foetal liver is also a source of HSCs, and, despite their relatively small number, it is possible to generate sufficient numbers of cells for transplantation in four weeks (Rollini et al. 2004), although, to our knowledge, it has not been proved that foetal liver HSCs can successfully transplant a human recipient. The phenotype of foetal liver HSCs changes with gestation age. For example, their differentiation potential decreases markedly with gestational age, and cells that expressed both CD34 and c-kit (CD117) were identified in the first-trimester foetal liver whereas the cells expressing more committed hepatic markers only appeared during the second trimester (Nava et al. 2005).

2.2. Extraembryonic tissue

2.2.1. Umbilical cord blood. Umbilical cord blood is now an established source of transplantable HSCs that have a greater proliferative capacity, lower immunological reactivity and lower risk of graft-versus-host disease (GVHD) than those derived from adult bone marrow (Broxmeyer et al. 1989; Yu et al. 2001; Ballen 2005; Schoemans et al. 2006; Brunstein et al. 2007; Hwang et al. 2007; Broxmeyer 2010). These cells are capable of repopulating bone following intra-bone injection of severe combined immunodeficiency mice (Mazur et al. 2003; Wang et al. 2003) and are used clinically as an alternative to adult bone marrow HSCs in some cases (Delaney et al. 2010). The cells are CD34+ and CD38– and their frequency is greater than that of bone marrow or peripheral blood following cytokine mobilization (Pappa & Anagnou 2009). It has also been demonstrated that cord blood contains MSCs (Weiss & Troyer 2006; Secco et al. 2008) that can support the in vitro expansion of HSCs and function as an accessory cell population for engraftment (Javazon et al. 2004; Wang, J. et al. 2004). The frequency of MSCs in umbilical cord blood is low however, with MSCs successfully isolated from only a third of all samples collected (Bieback et al. 2004).

Cord blood stem cells expressing baseline levels of ES cell markers such as Oct-4, Nanog, SSEA-3 and SSEA-4 have also been described (Zhao et al. 2006). Using a two-stage isolation approach whereby the erythrocytes in cord blood are lysed and the remaining cells are sorted using flow cytometry, it has been possible to isolate and enrich for a subpopulation of cells that are CXCR4+, CD133+, CD34+, Lin– and CD45–.

These cells have been described as very small embryonic-like (VSEL) stem cells because they are only 3–5 μm in diameter and express Oct-4, Nanog and SSEA-4 (Kucia et al. 2007; Zuba-Surma et al. 2010). Compared with MSCs, VSEL cells are smaller, have a large nucleus to cytoplasm ratio and open-type chromatin. As a similar population has also been
found in adult bone marrow, where they also express the same primitive markers, it has been hypothesized that VSEL cells are related to a population of early PGCs that are retained during development (Kucia et al. 2006).

2.2.2. Wharton’s jelly. Wharton’s jelly is the connective tissue surrounding the umbilical vessels and includes the perivascular, intervascular and subamnion regions (Troyer & Weiss 2008). MSCs have been isolated from all three regions and it remains unclear whether they represent distinct cell populations (Karahuyscoinlu et al. 2007). The cells have been given different names by different groups (Troyer & Weiss 2008). MSCs isolated from Wharton’s jelly share similar properties with other cord blood MSCs as well as adult bone marrow MSCs on the expression of markers, differentiation potential and cytokine production (McElreavey et al. 2004; Anzalone et al. 2010). Wharton’s jelly MSCs can also be induced to differentiate into cells similar to neural, expressing neuron-specific enolase and other neural markers (Mitchell et al. 2003; Anzalone et al. 2010).

2.2.3. Amniotic fluid. In recent years, amniotic fluid has emerged as a major source of putative pluripotent stem cells that avoid many of the problems associated with ES cells such as their non-suitability for autologous use, their capacity for tumour formation and the ethical concerns they raise.

In humans, the amniotic fluid starts to appear at the beginning of week 2 of gestation as a small film of liquid between the cells of the epiblast. The fluid expands separating the epiblast (i.e. the future embryo) from the amnioblasts (i.e. the future amnion), thus forming the amniotic cavity (Miki & Strom 2006). The origin of amniotic fluid cells is still very much debatable (Kumisaki et al. 2007; Cannanzi et al. 2009). However, what is known is that the majority of cells present are terminally differentiated and have limited proliferative capacity (Gosden & Brock 1978; Siegel et al. 2007). However, a number of studies have demonstrated the presence of a subset of cells with a proliferative and differentiation potential (Torricelli et al. 1993; Streubel et al. 1996).

A variety of different types of stem cells have been isolated and characterized from amniotic fluid. These include cells found in mid-gestation expressing the haematopoietic marker CD34 (Da Sacco et al. 2010) as well as cells with mesenchymal features, able to proliferate in vitro more rapidly than comparable foetal and adult cells (Kaviani et al. 2001; in’t Anker et al. 2003a,b; Nadri & Soleimani 2007; Roubelakis et al. 2007; Sessarego et al. 2008). Amniotic fluid MSCs are negative for haematopoietic markers such as CD45, CD34 and CD14 (Prusa & Hengstschlager 2002; in’t Anker et al. 2003a,b; Prusa et al. 2003; Tsai et al. 2004). Despite their high proliferation rate, these cells display a normal karyotype when expanded in vitro and do not form tumours in vivo (Sessarego et al. 2008). They exhibit a broad differentiation potential towards mesenchymal lineages (in’t Anker et al. 2003a,b; Kolambkar et al. 2007; Nadri & Soleimani 2007; Tsai et al. 2007).

De Coppi et al. isolated c-kit-positive (CD117) cells that represent about 1 per cent of cells present in second-trimester amniotic fluid. These cells were named amniotic fluid stem (AFS) cells. They can be cultured without feeders, double in 36 h, are not tumorigenic, have long telomeres and retain a normal karyotype for over 250 population doublings (De Coppi et al. 2007a). Cultured human AFS cells are positive for ES cell (e.g. Oct-4, Nanog and SSEA-4) and mesenchymal cell markers such as CD90, CD105 (SH2), CD73 (SH3/4) and several adhesion molecules (e.g. CD29 and CD44; Tsai et al. 2006; Chambers et al. 2007; De Coppi et al. 2007a). Furthermore, it was possible to generate clonal human lines from these cells, verified by retroviral marking, which were capable of differentiating into lineages representative of all three EG layers. Almost all clonal AFS cell lines express Oct-4 and Nanog, markers of a pluripotent undifferentiated state (De Coppi et al. 2007a). c-kit+ Lin- cells from human and mouse amniotic fluid display a multi-lineage haematopoietic potential in vitro and in vivo, despite having low or negative CD34 expression (Ditadi et al. 2009). c-kit-positive human AFS cell lines can also form embryoid bodies (EB) with an incidence of 18–82%. EB formation was accompanied by a decrease in Oct-4 and Nodal and the induction of differentiation markers such as Pax 6, Nestin (ectodermal), GATA 4 and HBE1 (mesodermal) (Valli et al. 2010). The potential for a wide range of regenerative medicine and tissue engineering applications as well as their relatively few ethical concerns makes them an attractive source for cell therapy; particularly considering that a bank of 100,000 amniotic fluid specimens could potentially supply 99 per cent of the US population with a perfect match for transplantation (Atala 2009).

2.2.4. Placenta. The placenta is a fetomaternal organ involved in maintaining foetal tolerance and allows nutrient uptake and gas exchange with the mother, but also contains a high number of progenitor cells or stem cells (Parolini et al. 2010). It has two sides: one foetal (amnion and chorion) and one maternal (decidua). The amnion membrane contains two cell types: the amniotic epithelial cells (AECs) derived from the epiblast; and amniotic mesenchymal cells derived from the hypoblast. The chorion layer consists of cytotrophoblasts and syncytiotrophoblasts that are derived from the outer layer of the blastocyst (trophoblastoderm) (Ilancheran et al. 2009; Insuasti et al. 2010).

The availability, phenotypic plasticity and immunomodulatory properties of placenta-derived progenitor/ stem cells are useful characteristics for cell therapy and tissue engineering. Cells can be isolated during ongoing pregnancy using minimally invasive techniques such as chorionic villus sampling (CVS) and placental tissues are readily available at delivery for allogeneic or autologous use. Cells that have been isolated from placenta include the human AECs, human amniotic mesenchymal stromal stem cells (AMSCs), human chorionic mesenchymal/stromal stem cells (CMSCs), human chorionic trophoblastic cells and HSCs (Parolini et al. 2008).
Mesenchymal placenta cells are plastic adherent, share a similar immunophenotype and have lineage differentiation potential. They express stromal markers such as CD166, CD105, CD73, CD90 and others, while they are negative for the haematopoietic markers CD14, CD34 and CD45 (Igura et al. 2004; Sudo et al. 2007). Additionally, they express pluripotency markers such as SSEA3, SSEA4, Oct-4, Nanog, Tra-1-60 and Tra-1-81 (Portmann-Lanz et al. 2007). They lack or have very low expression of MHC class I antigens and do not express MHC class II antigens or the T-cell co-stimulatory molecule B7, giving the cells immunomodulatory properties (Bailo et al. 2004; Miao et al. 2006).

2.2.4.1. Amniotic epithelial cells. AECs are commonly isolated from the amniotic membrane using digestive enzymes. The cells are plastic adherent and grow under MSC conditions, i.e. Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10–20% FBS, with the addition of growth factors such as epidermal growth factor (EGF) (Miki et al. 2010). c-Kit and CD90 (Thy-1) expression is either negative or at a low level (Miki et al. 2005; Miki & Strom 2006). However, evidence suggests that they express pluripotency markers and have the ability to form xenogeneic chimera with mouse ES cells in vitro (Tamagawa et al. 2004). The cells have subsequently been differentiated in vitro into cell types from all three germ layers (Miki et al. 2005; Miki & Strom 2006; Ilancheran et al. 2007; Parolini et al. 2008). However, in contrast with ES cells, Miki et al. (2005) demonstrated that human AE cells did not form gross or histological tumours up to seven months post-transplantation in SCID/Beige mice.

2.2.4.2. Amnion-derived mesenchymal stromal cells. Both amnion and chorion MSCs have been extensively characterized and can be isolated throughout gestation from first trimester to delivery. Both cells share common characteristics, such as plastic adherence, and, despite limited proliferation capacity, show in vitro differentiation down the osteogenic, adipogenic, chondrogenic and neurogenic lineages, although some report and show in vivo multi-organ engraftment capacity. In addition, the volume of term placenta makes it an attractive source of stem cells, as on average human term placenta weighs more than 590 g (Bolisetty et al. 2002). Human placenta contains various types of cells of different developmental origin. Cells of the chorion and decidua are derived from trophoderm, while amnion is derived from the epiblast of the developing embryo (Crne & Cheung 1988).

MSCs have been successfully isolated from first-, second- and third-trimester placental compartments, including the amnion, chorion, decidua parietalis and decidua basalis (in’t Anker et al. 2004; Portmann-Lanz et al. 2006; Soncini et al. 2007; Poloni et al. 2008), and represent less than 1 per cent of cells present in the human placenta (Zhang et al. 2004; Alviano et al. 2007). First-trimester placenta stem cells generally grow faster than those found in the third trimester (Portmann-Lanz et al. 2006), and the majority of term placenta-derived stem cells are found at the quiescent G0/G1 phase of the cell cycle.

2.2.4.3. Chorion-derived stem cells. CMSCs have been isolated during pregnancy (CVS) or from the term placenta at delivery. The immunophenotype of term placenta cells is similar to that of adult bone marrow MSCs, although renin and flt-1 have been shown to be expressed uniquely in placenta MSCs (Fukuchi et al. 2004). Similarly to human amnion isolated from term placenta, term chorion MSCs successfully engraf in neonatal swine and rats and failed to induce a xenogeneic response (Bailo et al. 2004), indicating that these cells may have an immunoprivileged status consistent with their low level of HLA I and absence of HLA II expression (Kubo et al. 2001). Of relevance for perinatal autologous and allogeneic therapy, chorion MSCs can be successfully isolated from chorionic vili during first-trimester gestation. The chorionic villi do not express typical MSC cell surface antigens and have the capacity to differentiate into lineages of the three germ layers, with a subset of cells expressing the pluripotency markers Oct-4, ALP, Nanog and Sox2 (Spitalieri et al. 2009). In addition to differentiating into adipogenic, chondrogenic and osteogenic cells in vitro (Portmann-Lanz et al. 2006; Ilancheran et al. 2007), they can also differentiate into cells with some characteristics of hepatocytes in vitro and have the ability to store glycogen (Chien et al. 2006; Huang 2007; Tamagawa et al. 2007). Differentiation into other cell types such as endothelial cells (Alviano et al. 2007), cardiomyocytes (Zhao et al. 2005), neurons, oligodendrocytes and glial cells (Miao et al. 2006; Yen et al. 2008; Portmann-Lanz et al. 2010) has also been reported, but these findings have mostly relied on upregulation of tissue-specific markers without providing robust functionality tests.

3. THERAPEUTIC APPLICATIONS OF FOETAL STEM CELLS

Regenerative medicine aims to restore diseased or damaged tissue using the body’s own cells in order to overcome the shortage of donated organs and the risk associated with their rejection (Atala 2009). The use of stem cells from foetal, cord blood and extraembryonic tissues for regenerative medicine applications has increased over the past few years. However, the surprising picture that has emerged is that cell replacement is probably not the major mechanism by which cell therapy confers functional benefit. Rather, stem cells act beneficially by exerting trophic effects on host tissues. Moreover, because they can be expanded to very large numbers compared with adult stem cells, they are ideal candidates for seeding scaffolds and matrices used for tissue engineering applications such as the repair of hollow and solid organs. In this section, we will consider some of the therapeutic applications FSCs have been put to in pre-clinical studies for repairing diseased or damaged tissue as well as in cancer treatment (summarized examples are listed in table 2).
<table>
<thead>
<tr>
<th>cell type</th>
<th>cell origin</th>
<th>treated recipient</th>
<th>regenerated tissue</th>
<th>method of cell delivery</th>
<th>disease treated</th>
<th>outcome</th>
<th>references</th>
</tr>
</thead>
<tbody>
<tr>
<td>foetal tissues</td>
<td>human</td>
<td>oim mice</td>
<td>bone</td>
<td>intrauterine</td>
<td>osteogenesis imperfecta (OI)</td>
<td>transplantation resulted in decrease in fractures, increased bone strength, thickness and length. Transplanted cells were found clustered in areas of active bone formation</td>
<td>Guillot et al. (2008a) and unpublished data</td>
</tr>
<tr>
<td>blood and bone</td>
<td>postnatal scid/ mdx dystrophic mouse model/ prenatal Mdx dystrophic mice</td>
<td>muscle</td>
<td>intra-arterial delivery into post-natal mice/ intrauterine transplantation using intramuscular, intravascular and intraperitoneal delivery of cells into pre-natal mice</td>
<td>Duchenne muscular dystrophy (DMD)</td>
<td>galectin-1-exposed foetal MSCs formed fourfold more human muscle fibres in post-natal scid/ mdx mice than non-stimulated cells. In pre-natal immunocompetent mdx mice, intravascular delivery led to demise of mice, intraperitoneal delivery led to systemic spread of cells and intramuscular injections resulted in local engraftment. The cells differentiated at low level into skeletal and myocardial muscle but not at a curative level</td>
<td>Chan et al. (2006, 2007)</td>
<td></td>
</tr>
<tr>
<td>liver</td>
<td>human</td>
<td>human foetus diagnosed with severe OI</td>
<td>bone</td>
<td>intrauterine</td>
<td>OI</td>
<td>cell engraftment of bone despite the donor cells being HLA-mismatched with recipient. Bone histology showed regularly arranged and configured bone trabeculae</td>
<td>Le Blanc et al. (2005)</td>
</tr>
<tr>
<td>pancreas</td>
<td>human</td>
<td>sheep</td>
<td>pancreas</td>
<td>intrauterine</td>
<td>type II diabetes</td>
<td>the cells functionally engrafted in the pancreas in 50% of sheep. The engrafted cells differentiated and were able to secrete insulin</td>
<td>Ersek et al. (2010)</td>
</tr>
<tr>
<td>extraembryonic</td>
<td>human</td>
<td>human</td>
<td>bone</td>
<td>systematic administration</td>
<td>malignant and non-malignant blood disorders</td>
<td>used in the treatment of leukaemia/ cancer, Fanconi anaemia, severe aplastic anaemia, myelodysplastic syndrome and other disorders</td>
<td>Broxmeyer (2010)</td>
</tr>
<tr>
<td>cord blood</td>
<td>human</td>
<td>human mouse</td>
<td>pancreas</td>
<td>intra peritoneal</td>
<td>type II diabetes</td>
<td>cord blood stem cells introduced into streptozotocin-induced diabetic mouse model differentiated into functional insulin-producing cells and eliminated hyperglycaemia</td>
<td>Zhao et al. (2006)</td>
</tr>
</tbody>
</table>

(Continued.)
<table>
<thead>
<tr>
<th>cell type</th>
<th>cell origin</th>
<th>treated recipient</th>
<th>regenerated tissue</th>
<th>method of cell delivery</th>
<th>disease treated</th>
<th>outcome</th>
<th>references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wharton’s Jelly</td>
<td>human</td>
<td>rat</td>
<td>brain</td>
<td>injection into the brain striatum</td>
<td>Parkinson’s disease</td>
<td>the cells ameliorated apomorphine-induced rotations in a rat model of Parkinson’s disease</td>
<td>Weiss et al. (2006)</td>
</tr>
<tr>
<td>amniotic fluid</td>
<td>sheep</td>
<td>sheep</td>
<td>muscle</td>
<td>cells seeded on collagen hydrogel and placed on intestinal cellular graft</td>
<td>partial diaphragmatic replacement</td>
<td>Parkinson’s disease diaphragmatic hernia recurrence was significantly higher in animals that did not receive the cell grafts</td>
<td>Fuchs et al. (2004)</td>
</tr>
<tr>
<td>rat</td>
<td>rat</td>
<td>smooth muscle</td>
<td>muscle</td>
<td>cells injected into site of injury</td>
<td>wound healing of injured bladder</td>
<td>cells prevented cryo-injury-induced hypertrophy of surviving bladder smooth muscle cells but failed to differentiate specifically to smooth muscle</td>
<td>De Coppi et al. (2007a)</td>
</tr>
<tr>
<td>mouse</td>
<td>mouse</td>
<td>brain</td>
<td>intra ventricular injection</td>
<td>focal cerebral ischaemia reperfusion injury</td>
<td>lung injury</td>
<td>the cells significantly reversed neurological deficits in the treated animals</td>
<td>Rehni et al. (2007)</td>
</tr>
<tr>
<td>human</td>
<td>mouse</td>
<td>lung</td>
<td>systematic administration</td>
<td>lung injury</td>
<td>AFS cells exhibited a strong tissue engraftment in a mouse lung injury model and expressed specific alveolar and bronchiolar epithelial markers</td>
<td>Carraro et al. (2008)</td>
<td></td>
</tr>
<tr>
<td>human</td>
<td>rat</td>
<td>heart</td>
<td>intramyocardial injection</td>
<td>myocardial infarction</td>
<td>myocardial infarction</td>
<td>treated animals showed a preservation of the infarcted thickness, attenuation of left ventricle remodelling, a higher vascular density and general improvement of cardiac function</td>
<td>Yeh et al. (2010)</td>
</tr>
<tr>
<td>placenta</td>
<td>human amnion AEC</td>
<td>mouse</td>
<td>brain</td>
<td>intracranial injection</td>
<td>Parkinson’s disease</td>
<td>cells transplanted into a mouse model of Parkinson’s disease differentiated into neuron, astrocyte and oligodendrocyte and promoted endogenous neurogenesis</td>
<td>Kong et al. (2008)</td>
</tr>
<tr>
<td>human amnion</td>
<td>human amnion MSC</td>
<td>rat</td>
<td>heart</td>
<td>intramyocardial injection</td>
<td>myocardial infarction</td>
<td>cells integrated into normal and infarcted rat cardiac tissue where they differentiated into cardiomyocyte-like cells and resulted in considerable improvements to ventricular function, capillary density and scar tissue</td>
<td>Zhao et al. (2005), Ventura et al. (2007)</td>
</tr>
</tbody>
</table>
3.1. Bone
Osteogenesis imperfecta (OI) is characterized by osteopenia and bone fragility due to abnormal collagen production caused by mutations in the α chains of collagen type I. First-trimester allogeneic HLA-mismatched male foetal liver MSCs were transplanted into a female foetus diagnosed with severe OI. Bone biopsy showed regularly arranged and configured bone trabeculae and no adverse immune reaction was observed (Le Blanc et al. 2005). Using a mouse model of OI, our group has shown that in utero transplantation of foetal blood MSCs ameliorated the disease phenotype, producing a clinically relevant two-thirds reduction in fracture incidence along with an improvement in bone structure and mechanical properties (Guillot et al. 2008a). Foetal bone marrow MSCs loaded on a highly porous scaffold were able to reach confluence within the scaffold more quickly than umbilical cord and adult MSCs. Furthermore, they demonstrated higher in vitro and in vivo osteogenic differentiation capacity, highlighting their suitability for bone tissue engineering applications (Zhang et al. 2009).

3.2. Muscle
Duchenne muscular dystrophy (DMD) is an X-linked myopathy affecting one in 3500 boys. The main genetic defect leads to absence of dystrophin, resulting in muscle damage and wasting. The affected boys become wheelchair-bound by 12 years of age and succumb to respiratory failure or cardiomyopathy by the third or forth decade of life (Manzur & Muntoni 2009). The main animal model for studying DMD is the mdx mouse that has a premature stop codon resulting in a termination in exon 23 of the dystrophin gene (Grounds et al. 2008a). Galectin-1-treated foetal blood and bone marrow MSCs, transplanted into postnatal severe combined immunodeficiency/dystrophic mice (scid/mdx) as well immunodeficient mice whose muscle was induced to regenerate by cryodamage, formed fourfold more human muscle fibres than non-stimulated MSCs (Chan et al. 2006). Intrauterine transplantation of foetal MSCs into immunocompetent E14-16 mdx mice resulted in widespread long-term engraftment in multiple organs with a predilection for muscle compared with non-muscle tissues. However, the engraftment level observed (0.5–1%) falls significantly below the levels required for functional improvement in DMD. The low engraftment might be linked to an absence of muscle pathology at the time of transplantation (Chan et al. 2007).

AFS cells have been used in an ovine model of diaphragmatic hernia: repair of the muscle defect using grafts engineered with autologous mesenchymal amniocytes leads to better structural and functional results than equivalent foetal myoblast-based and acellular grafts (Fuchs et al. 2004; Kuriyama et al. 2006). In a rat model of bladder cryo-injury, AFS cells also show the ability to differentiate into smooth muscle and to prevent the compensatory hypertrophy of surviving smooth muscle cells (De Coppi et al. 2007b).

Human AFS cells, transplanted into an immune-competent and ciclosporin immune-suppressed rat model of myocardial infarction, were rejected probably because of the expression of B7 co-stimulatory molecules as well as macrophage marker CD68. Furthermore, chondro-osteogenic cell masses were observed in the infarcted hearts of some of the transplanted animals (Chiavegato et al. 2007). However, in another study it was found that these masses occurred independently of AFS cell injection and that the cells did not increase the presence of these masses (Delo et al. 2010).

Human AMSCs are able not only to express cardiogenic-specific genes under specific culture conditions, but also to integrate into normal and infarcted rat cardiac tissue where they differentiate into cardiomyocyte-like cells (Zhao et al. 2005). The cells result in considerable improvements to ventricular function, capillary density and scar tissue (Ventura et al. 2007) and similar findings have been reported using native rat AMSCs (Fujimoto et al. 2009). Stem cells derived from pre-natal chorionic villi were also successfully engineered into a living autologous heart valve, which could have postnatal applications for repairing congenital cardiac malformations (Schmidt et al. 2006).

3.3. Kidney
In a mouse model of collagen deficiency characterized by abnormal collagen deposition in renal glomeruli (Phillips et al. 2002; Brodeur et al. 2007), we recently showed that intrauterine transplantation of foetal blood MSCs led to a reduction of abnormal homotrimeric collagen type I deposition in the glomeruli of 4–12 week old collagen2-deficient mice. Furthermore, we showed that the damaged kidneys preferentially recruited donor cells in the glomeruli. The study supports the feasibility of pre-natal treatment for renal diseases such as Alport syndrome and the polycystic kidney diseases (Guillot et al. 2008b).

Acute tubular necrosis could also be potentially treated with foetal cells. Human AFS cells injected into an immunodeficient mouse model of the disease decreased the number of damaged tubules and reduced apoptosis. The cells also promoted the proliferation of tubular epithelial cells and appeared to have a beneficial immunomodulatory effect (Perin et al. 2010).

3.4. Lung
Human AFS cells injected into the tail vein of nude mice following hyperoxia injury show the capacity to home to the lung and engraft. They also expressed specific alveolar and bronchiolar epithelial markers (e.g. TFF1, SPC and CC10; Carraro et al. 2008).

AECs and AMSCs from either human or mouse have been used in a mouse model of bleomycin-induced lung injury and shown to cause a reduction in severity of lung fibrosis. This occurs regardless of cell source (allo- or xenogeneic) or administration route (systemic, intravenous or intraperitoneal; local, intratracheal; Cargnoli et al. 2009).

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3.5. Liver
Second-trimester foetal liver cells, enriched for CD326 (hepatocyte progenitor marker) and labelled with Tc-d,1-hexamethylenepropylene-amine oxime (Tc-HMPOA) were infused into the hepatic artery of 25 patients with end stage liver cirrhosis. The cells restored the lost liver function in the patients without provoking an immune reaction, probably because of the absence of HLA class II expression of the infused cells (Khan et al. 2010).

Human AECs can secrete albumin in culture and, when β-galactosidase-tagged AECs were transplanted into immunodeficient mice, the cells integrated into the liver parenchyma and could be detected until day 7 post transplant (duration of study; Sakuragawa et al. 2000). In another study, the engrafted cells resulted in the detection of human α-1 antitrypsin in the serum of recipient animals, confirming that AECs are capable of performing this important hepatic function in vivo (Miki & Strom 2006).

3.6. Brain
Human Wharton’s jelly MSCs ameliorate apomorphine-induced behavioural deficits in a hemiparkinsonian rat model (Weiss et al. 2006). There was a significant decrease in apomorphine-induced rotations at four weeks continuing up to 12 weeks post transplantation in Parkinson’s disease (PD) rats that received human Wharton’s jelly MSCs transplants compared with the PD rats that received a sham transplant. The behavioural findings correlated with the numbers of tyrosine hydroxylase-positive cell bodies observed in the mid-brain following sacrifice at 12 weeks, indicating a ‘rescue from a distance’ phenomenon. One explanation for this effect may be that Wharton’s jelly MSCs synthesize glial cell-derived neurotrophic factor (GDNF), a potent survival factor for dopaminergic neurons, as well as other trophic factors such as vascular endothelial growth factor (VEGF) and ciliary neurotrophic factor. In another report, Wharton’s jelly MSCs were first induced towards dopaminergic neurons, as well as neuronal and astrocytic lineages (Rachakatla et al. 2010). Of these, MSCs expressing human interferon beta and used to deliver a secretable trimeric form of tumour necrosis factor-related apoptosis-inducing ligand (stTRAIL), via adenoviral transduction mediated by cell-permeable peptides, to human glioma cells in vitro demonstrated a significant decrease in tumours and not in other tissues. They were engineered to express human interferon beta and were administered intravenously into SCID mice bearing tumours. This treatment significantly reduced the tumour burden (Rachakatla et al. 2007). Similarly, human umbilical cord blood-derived MSCs (UCB–MSCs) have been used to deliver a secretable trimeric form of tumour necrosis factor-related apoptosis-inducing ligand (stTRAIL), via adenoviral transduction mediated by cell-permeable peptides, to human glioma cells in nude mice. The genetically modified cells showed
greater efficacy in terms of inhibiting tumour growth and prolonging the survival of glioma-bearing mice compared with direct injection of adenovirus encoding the sTRAIL gene into the tumour mass (Kim et al. 2008).

4. USE OF FSCs FOR REPROGRAMMING

The major advantage of human ES cells over other stem cell types is their capacity to differentiate into lineages from the three germ layers. However, there are two major objections to their use. The moral objection is that their derivation requires the destruction of embryos. The practical objection is that they have a limited clinical application as they can only be used in an allogeneic fashion. This is also true for some foetal tissues such as liver and bone marrow. However, extraembryonic tissues can be used autologously.

Thus, it was seen as advantageous to develop a method of creating ES-like cells from somatic cells. Early attempts at achieving this were nuclear transplantation and fusion of somatic cells and ES cells (Jaenisch 2009). However, a considerable step forward was the generation of the induced pluripotent stem (iPS) cells. The production of iPS cells with quasi-identical genetic and functional properties offers the possibility to bypass both moral conflicts and different genetic background inherent to the technologies mentioned above. iPS are developed from a non-pluripotent cell,usually an adult somatic cell, by causing a forced expression of several genetic sequences above. iPS are developed from a non-pluripotent cell, usually an adult somatic cell, by causing a forced expression of several genetic sequences and were first produced in 2006 by Takahashi & Yamanaka from mouse somatic cells. The key genes Oct-4, the transcription factor Sox2, c-Myc protooncogene protein and Klf4 (Krueppel-like factor 4) were sufficient to reprogramme fibroblasts to cells closely resembling ES cells (Takahashi & Yamanaka 2006; Takahashi et al. 2007).

Despite the high similarity between iPS and ES cells, tumour formation in iPS cell chimeric mice was high, presumably because of the expression of c-Myc in iPS cell-derived somatic cells (Maherali et al. 2007). Thomson et al. showed that c-Myc was not necessary as they were able to generate iPS cells using Oct-4, Sox2, Nanog and Lin28 using a lentiviral system (Yu et al. 2007). More recently, it was shown that human fibroblasts cells can be used to generate iPS cells if transduced with Oct-4 and Sox2 only in the presence of valproic acid (Huangfu et al. 2008).

Terminally differentiated cells might not be the ideal candidate for iPS cell generation because a greater number of steps could be required to ‘reprogramme’ their genome than somatic stem cells. Kim et al. (2009a,b) have shown that it is possible to generate iPS cells by transducing adult mouse neural stem cells (NSCs) and human foetal NSCs with Oct-4 only. Furthermore, when human adipose MSCs were used, they were reprogrammed more efficiently than fibroblasts (Sun et al. 2009).

Few reports have also emerged on the use of cord and extra ES cells for iPS generation. Galende et al. used terminally differentiated amniotic fluid cells as candidates for reprogramming and found that the iPS cell colonies were generated twice as fast, yielding nearly a 200 per cent increase in number compared with cultured adult skin cells. However, they did not provide absolute efficiency data (Galende et al. 2009). Li et al. reprogrammed human amniotic fluid-derived cells (hAFDCs) with efficiencies varying from 0.059 to 1.525 per cent. The iPS colonies generated expressed pluripotency markers such as Oct-4, Sox2 and SSEA 4 and they maintained a normal karyotype (Li et al. 2009). Wharton’s jelly cells and term placenta cells have also been used, giving reprogramming efficiencies of 0.4 per cent and 0.1 per cent, respectively, which compare rather well with efficiencies reported for adult fibroblasts (less than 0.01%). The generated iPS cell colonies expressed several pluripotency markers, formed EB and, when injected into nude mice, they generated teratoma-containing derivatives of the three germ layers (Cai et al. 2010). Giorgetti et al. selected CD133+/CD34−, human CD133+ bone marrow-derived cord blood cells (expressing pluripotency markers and capable of forming EB and teratomas) were generated in two weeks whereas no colonies were obtained with control keratinocytes or fibroblasts using the two factors only (Giorgetti et al. 2009, 2010).

CD133+/CD34− cord blood cells and foetal NSCs already have a baseline level expression of Oct-4 and Sox2 that, along with a generally more permissive chromatin organization, might be the key to their greater reprogramming efficiency compared with adult cells.

5. CONCLUSION

Human FSCs can be isolated from foetal organs or extraembryonic sources. They have the advantage of rapid proliferation, stable karyotype and low or negligible immunogenicity. Unlike ES cells, they do not form teratomas in vivo and have far fewer ethical concerns as they are mostly obtained from tissues that would otherwise be discarded. Many of these cells seem to express some of the same pluripotency markers found in ES cells, a feature largely absent from most adult-derived stem cells. They also have the further advantage over adult stem cells of senescing much later and being more readily amenable to genetic modification.

All of these features make them valuable for potential therapy applications. Thus far they have been used in pre-clinical settings to treat a variety of diseases such as osteogenesis imperfecta, congenital diaphragmatic hernia, Parkinson’s disease and cancer with encouraging results. Finally, their usefulness for iPS generation is very likely to expand their future clinical use even further.

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