2

Human Cord Blood-Derived Stem Cells in Transplantation and Regenerative Medicine

Cornel Iancu et al.*
"Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca
Romania

1. Introduction

Regenerative medicine can be defined as the process of restoring diseased or damaged tissue by replacing it with biological substitutes and this issue represents one of the main concerns of medicine. Stem cells are likely to be used in the future for cardiac, neurologic, hepatic, orthopaedic and other applications. The stem cell populations have proved to be highly proliferative lately as isolated from a variety of embryonic, foetal and adult tissues. Their increased capacity to self-renew and to unlimitedly differentiate into derivates of all germ layers in vitro and in vivo have rendered embryonic stem cells (ESCs) the main concern of tissue engineering research and regenerative medicine (Hyslop et al., 2005). However these cell lines originate from the inner cell mass of human blastocysts. Thus, the relation of ESCs to the human blastocyst will always stir ethical, moral and emotional debate over their use in research. Moreover, besides these ethical and political issues, another drawback concerning their clinical application is their lack of accessibility, technique difficulties in purification and manipulation as well as the risk for teratoma development (Fan et al., 2011). Consequently, though ESC therapy has just come into attention and there still are unknowns, its success might result in it being followed by alternative stem cell therapies (Rogers & Casper, 2004).
Adult stem cells (ASCs) originate in a mature organism, including the brain, fat, skin, kidney, peripheral blood and bone marrow and they contribute to maintaining and repairing tissue that contain them. The adult-derived stem cells are also believed to have the ability to differentiate into tissues different from their tissue of origin (Krause et al., 2001; Jiang et al, 2002). Different from ESCs, ASCs can be easily harvested from various tissues, such as skin (Riekstina et al., 2008 as cited in Fan et al., 2011), bone marrow (BM) (Gastens et al., 2007 as cited in Fan et al., 2011) and adipose (Keiser et al., 2007 as cited in Fan et al., 2011), and might be employed in the clinical treatment of disorders of vulnerable vital organs. ASCs have the advantage that allows for small samples of tissues or even the patient's own cells to be used for implantation, avoiding problems of tissue rejection. Moreover, adult cells do not involve the typical ethical issues of embryonic research. In spite of these advantages, they are not the first choice and this is because of several reasons such

* Ioana Ilie, Lucian Mocan, Carmen Georgescu, Razvan Ilie, Ileana Duncea, Teodora Mocan, Dana Iancu, and Florin Zaharie

as their limited numbers, diminished growth and differential capacities age-dependent as well as invasive harvesting procedures (Roobrouck et al., 2008).
Compared to the extensive research on ESCs and ASCs, fetal stem cells have only recently come into attention (Fan et al., 2011). The transplant of fetal tissue has recently become a research therapy for chronic degenerative disease such as Parkinson's disease and insulin-dependent diabetes mellitus (DM). However, this method is widely opposed as fetal tissue originates from elective abortion (Rogers & Casper, 2004). However, the following two distinct sources have lately generated putative stem cells: the fetus proper (including fetal bone marrow (Chang et al., 2006 as cited in Fan et al., 2011), lung (Fan et al., 2005 as cited in Fan et al, 2011), spleen, liver (In't Anker et al., 2003 as cited in Fan et al., 2011), pancreas (Hu et al., 2003 as cited in Fan et al., 2011) and peripheral blood (Yu et al., 2004 as cited in Fan et al., 2011) and the supportive extra-embryonic structures (such as umbilical cord blood (Lu et al., 2005 as cited in Fan et al., 2011), umbilical cord (UC) (Fan et al. 2005 as cited in Fan et al., 2011), amniotic fluid (AF) (Mareschi et al., 2009 as cited in Fan et al., 2011), placenta (In't Anker et al., 2004 as cited in Fan et al., 2011) and amnion (Bilic et al., 2008 as cited in Fan et al., 2011), respectively.
New efficient stem cells sources would have to exhibit a similary potential to that of ESCs and to be highly proliferative or at least easy to harvest in large numbers. Out of these, the preferred ones are the BM-derived stem cells or UC-derived stem cells. There are two main cell types within the BM: mesenchymal, which are highly proliferative and show a propensity for multi-lineage differentiation (Rogers & Casper, 2004) and haematopoietic, also promising as multipotential stem cell (Kakinuma, 2003; Hao, 2003 as cited in Rogers & Casper, 2004). As mesenchymal cells and blood cells are likely to become potential alternatives to ESCs in some clinical situations, umbilical cord blood (UCB) and peripheral blood (PB) have started to be more and more regarded as sources of bone-marrow-like stem cells due to their easy harvesting compared to bone marrow (Rogers & Casper, 2004) Cord blood is already considered an important source for stem cells, mainly because, different from BM, there is no need for a perfect human leucocyte antigen (HLA) match, there is a lower risk of graft versus host disease (GVHD) with UCB transplantation than with BM transplantation and because UCB has more haematopoietic stem cells per volume than peripheral blood or bone marrow (Rogers & Casper, 2004; Barker & Wagner, 2003).
Consequently, the proliferation, the easy harvesting procedure, the advantages over embryonic and adults counterparts and no serious ethical issues are the main points that recommend the human cord blood (CB) as the most valuable solution against other stem cell sources, as detailed below.

2. The human umbilical cord: A source of stem cells

UCB has been widely considered an important stem cell source, because of its many pluses as compared with other stem cell sources. CB usually comes as a second choice after matched BM or PB but in the case of children suffering from leukaemia it represents the main stem cell source for transplant. (Stanevsky et al., 2009). Moreover, CB is also viewed as a primary stem cell source due to annual global human birth rate of more than 100 million a year. CB is easily and safely collected by CB banks and preserved as future therapeutic genetic material (Arien-Zakay et al., 2010). CB has been more and more considered as good alternative for embryonic stem cells lately (Figure 1 A), also because it has proved to contain populations of multipotent stem cells which are able to differentiate into a variety of cell types, including epithelial, endothelial, myotubes and neural [Harris & Rogers, 2007).

The human UC, a connecting tissue of extraembryonic origin, connects the fetus to the placenta. Developed during the fifth week of embryogenesis it reaches a final length of approximately 60–65 cm and a weight of about 40 g, and has a mean diameter of 1.5 cm in normal pregnancies. UC usually comprises two arteries and a vein, all immersed within a tissue that interconnects vessels- the so-called Wharton's jelly (WJ) and enclosed by a simple amniotic epithelium. WJ is a mucoid connective tissue abounding in proteoglycans and hyaluronic acid (HA), insulating and protecting umbilical vessels from torsion, compression, or bending. It is made of embedded stromal cells (Figure 1B). It thus assures a constant blood flow between fetus and placenta. (Fan et al., 2011; Malgieri et al., 2010). The blood remaining in the umbilical vein after birth is rich in hematopoetic stem and progenitor cells, and has been a successful alternative allogeneic donor source in the cure of a variety of pediatric genetic, hematologic, immunologic, and oncologic disorders (Broxmeyer et al., 1989; Gluckman et al., 1997; Han et al., 2003, Kim et al., 2002 as cited in Malgieri at al., 2010). Moreover, fresh cord blood is rich in non-hematopoietic stem cells, and also contains endothelial cells, mesenchymal stem cells (MSCs) and unrestricted somatic stem cells (USCC) (Kogler et al., 2004, 2006; Sensken et al., 2007; Greschat et al., 2008 as cited in Malgieri at al., 2010).

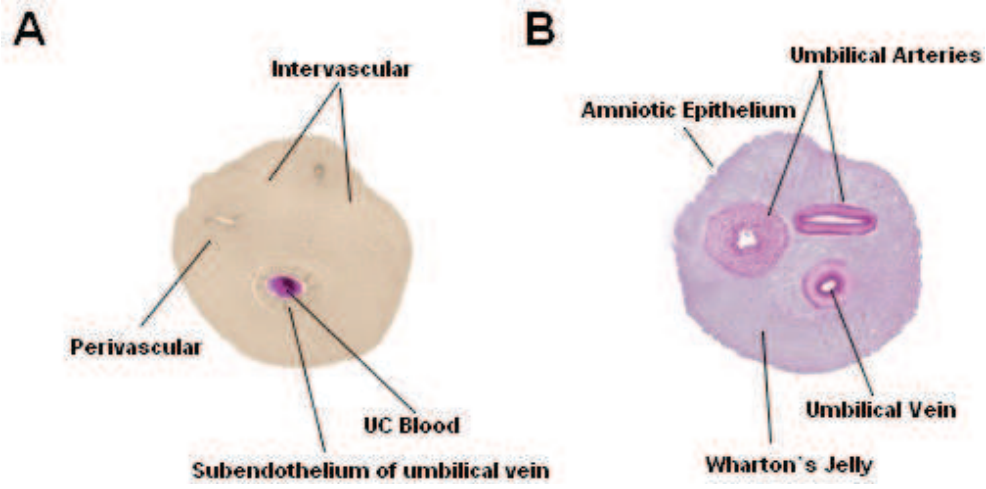


Fig. 1. Umbilical cord: cross section. (A)Localization of mesenchymal stromal cells. (B) Structure of umbilical cord wich contains two arteries and one vein surrounded by Wharton`s jelly and amniotic epithelium.

MSCs are refined as multipotent, undifferentiated cells that can self renew, regenerate mesenchymal tissues and blood cells and differentiate into several cell types such as chondrocyte, adipocyte, osteocyte, osteoblast, myocyte, cardiomyocyte, neuron-like cell as well as into insulin-producing cells (Jiang et al., 2002; Mareschi et al., 2006). There is not surprise, then, that they are the focus of regenerative medicine and tissue engineering (Arien-Zakay et al.,2010). Despite the fact that BM has been considered the main available source of MSCs (Pittenger et al., 1999), it is not always acceptable to use BM –derived cells because of the high degree of viral exposure, the significantly decreasing cell number and the proliferative/differentiation capacity along with age. Additionally, BM sampling

involves a painfully invasive procedure. Therefore, it is of utmost clinical importance in so far as the easy accessibility and the reduced morbidity are concerned to try to identify alternative sources of MSCs (Malgieri et al., 2010). Anyway, Mitchell et al. (Mitchell et al., 2003) successfully isolated the matrix cells from porcine and human umbilical cord by explants' culture only in 2003 and, in it was only in the same year that Romanov et al. (Romanov et al., 2003) managed to isolate mesenchymal like cells from sub-endothelial layer of human umbilical cord vein. Consequently, it is only since 2003 that the umbilical cord-derived MSCs have started to be extensively researched (Fan et al., 2003). Human UC blood-derived mesenchymal stem cells (hUCB-MSCs) exhibit ability similar to that of BM-MSCs for multi-lineage differentiation (Gang et al., 2004).
Consequently, UCB has proved a legitimate source for haematopoietic stem cell transplantation. Moreover, the development of research and scientific understanding of hUCB-MSCs will show that they are a really fascinating source to be used in stem cell therapy.

3. Isolation of MSCs from the umbilical cord

In recent years, several investigators published protocols for isolating MSCs from the UC tissue. These protocols have been developed and adapted based on the region of the cord from where the cells are harvested. Schematic illustration of harvesting protocols is given in Figure 2.

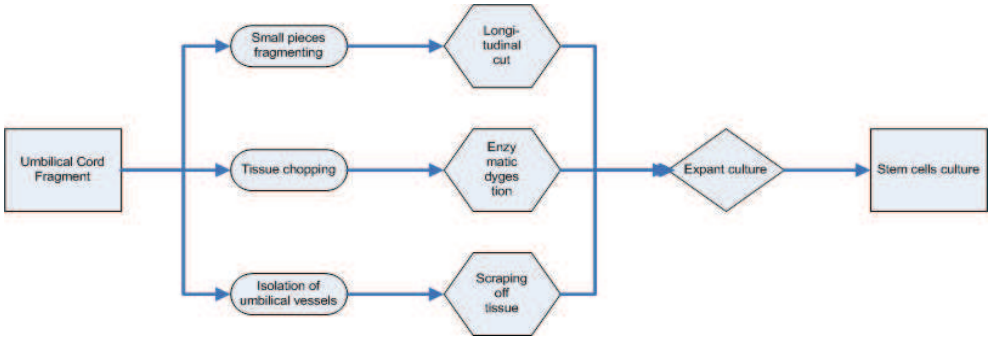


Fig. 2. Schematic illustration of various protocols used to isolate mesenchymal stem cells from the umbilical cord tissue.

The first step in the isolation procedure is represented by the removal of umbilical artery and vein followed by the splitting of the cord into smaller segments which are subsequently enzymatically digested (Weiss et al., 2006). Another techniques involve isolation methods without enzymatic digestions or removal of vessels (La Roca et al., 2009). Several methods were also described for the isolation of the cells from the perivascular tissue or the subendothelium of the umbilical vein (Covas et al., 2003).
Currently, the most used protocol is without enzymatic digestion in an explant culture approach without removal of umbilical vessels in order to isolate MSC-like cells from the whole UC tissue.
At the beginning, the blood from the umbilical vessels is removed by flushing phosphate buffered saline (PBS) through the artery and vein using a sterile syringe coupled to a small

tube. The next step is represented by the preservation of UC in a PBS medium enriched with 5g L^{-1} glucose, 50 ugmL^{-1} gentamicin, 2.5 ugmL^{-1} amphotericin B, 100 U.mL^{-1} penicillin, and 100 ugmL^{-1} streptomycin with the purpose of reducing the risk of contaminations. Then, the UC is serially cut first into 10-15 cm long segments and then into 0.5-0.8 cm^3 large pieces. During the isolation procedure, transfer medium is used to keep the cord and the minced pieces moist. Finally, the small pieces are transferred to 25 cm^3 flaks and incubated in a humidified atmosphere with 5% CO2 in a MEM supplemented with 15% of human serum and 50 ugm^{-1} gentamicin at 37°C which is changed every 48 hours. After 12 days, groups of adherent cells from single tissue pieces are observed. At this stage, the UC tissue is removed and the adherent cells are harvested by enzymatic treatment. The cell suspension is then centrifuged at 100 g for 10 min and the cells are resuspended in MEM supplemented with 10% human serum and 50 mgmL-1 gentamicin and subcultured at a density of 4000 cells cm~2. These culture conditions have demonstrated to support an optimal growth of the cells (Wobus et al., 2006).

Commonly, the isolated cells exhibited a high proliferation potential. For at least 20 population doublings, the cells can be expanded without loss of proliferative activity and viability (Figure 3). After approximately 50 population doublings the process of replicative senescence becomes common. At this point, UC-derived cells could be efficiently cryopreserved and revitalized in a cryo-medium containing 80% human serum, 10% culture medium, and 10 % DMSO.

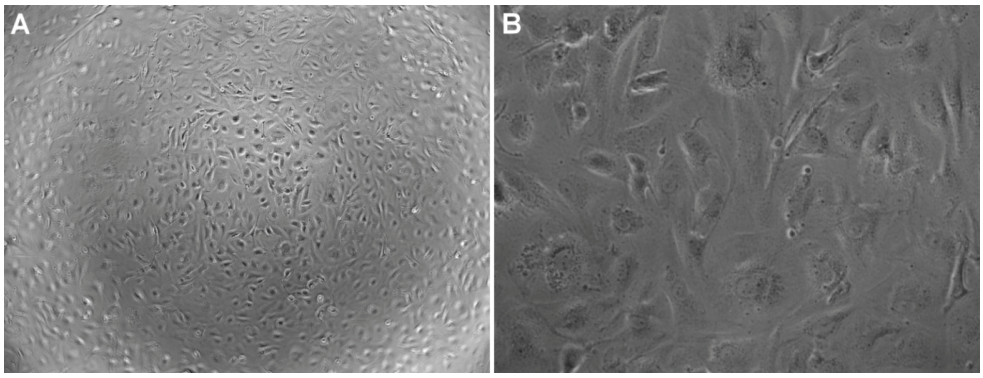


Fig. 3. Isolation of mesenchymal stem cells from umbilical cord: adherent growing monolayer of fibroblast-shaped cells after 21st days of culture (Magnification: A-10X, B-40X).

Depending on the isolation procedures, stem cells characteristics such as proliferation, differentiation potential and immunologic properties may be altered.

4. Characterization of UCSC

The evaluation of the nature of UCB HSC has demonstrated a higher proportion of primitive hematopoietic progenitors in UCB, with superior in vitro proliferative responses and in vivo engraftment capacity as compared to adult BM (Lewis et al., 2000; Wynter et al., 1999 as cited in Barker & Wagner, 2003) or PB (Barker & Wagner, 2003; Goldberg et al., 2007). Single UCB units can reconstitute entire lympho-hematopoietic systems in adults patients (Laughlin et al., 2001). In addition, UCB seems more tolerant of HLA mismatches, with less

GVHD. These unique biological features are further sustained by the fact that human UCB transplantation (UCBT) recipients show reliable myeloid and lymphoid hematopoietic reconstitution after myeloablative conditioning with greater than one log lesser nucleated cell (NC) dose than is used in BM transplantation (BMT).

Another unique characteristic of UCB is the reduced alloreactive response as compared with that seen with BMT, which is not fully explained. The median CD3+ cell dose of 8×106 kg−1 in UCB units makes it similar to a BM graft after modest T cell depletion (TCD). Such a T cell dose is fully capable of inducing significant GVHD, particularly in the setting of HLA mismatch. Therefore, reduced T cell number is insufficient to explain the relatively low incidence of severe GVHD with 1–2 antigen-mismatched UCBT. A more likely explanation is the functional differences reported with UCB lymphoid cells. These include higher levels of CD4+CD45RA+ cells, lower inflammatory cytokine production and responses, lower alloantigen and mitogen-specific T cell proliferation, a polyclonal T cell receptor repertoire, increased susceptibility to tolerance induction, and differences in NK cell and dendritic cell biology (Barker &Wagner, 2003; Canque et al., 2000).

In recent years, much excitement has been associated with studies that have suggested UCB does represent a source of multi-potent stem cells that could be used for generation of non-hematopoietic tissues. MSCs were isolated from the mononuclear cell fraction of CB using various criteria, including adherence to the surface of the culture medium, negative expression of hematopoietic markers such as CD34, CD133, CD45, CD14, and positive expression of mesenchymal markers such as CD90, CD105, CD73 (Flynn et al., 2007). The isolated UCB-MSCs were shown to differentiate into a wide range of cell types including bone, cartilage, cardiomyocytes, neural among others. Pre-clinical studies also revealed that MSCs from CB support ex vivo expansion and differentiation of haematopoietic stem/progenitor cells from CB. These MSCs improved the haematopoietic cells engrafted in non-obese diabetic/severe combined immune-deficient (NOD/SCID) mice (Huang et al., 2007). It is worth mentioning that there is no fully identified unique phenotype for MSCs derived from CB, as well as for MSCs from all other origins. This prevents reproducible isolation of MSC precursors with predictable potential for development, and their isolation and characterisation rely primarily on their ability to adhere to plastic and their ability to expand (Arien-Zakay et al., 2010). In the culture, the morphology of hUCB-MSCs demonstrates a typical MSCs immunophenothypic markers and fibroblastoid morphology. The absence of endothelial CD31 and leukocyte surface markers supports the involvement of UCB-MSCs as mesenchymal progenitors. Very important, as it was already mentioned, hUCB-MSCs are devoid of hematopoietic and endothelial markers such as CD14, CD28, CD31, CD33, CD34, CD45, CD56, CD133, HLA-DR, and for graft versus host disease CD80, CD86, CD40, which show that they are suitable for transplantation (Malgieri et al., 2010). The comparison between hUCB-MSCs and BM-MSCs showed that hUCB-MSCs share most of their immunophenotype with BM-MSCs, including a cluster of differentiating markers, neural markers and extracellular adhesion molecules as well as the cell cycle status, the adipogenic and osteogenic differentiation capacity and finally the cytokines and hematopoietic supportive function (Malgieri et al., 2010). Despite this, recent studies noted that there are still several differences between them. Firstly, the fibroblast colony-forming units (CFUF) frequency was significantly higher in UCB derived nucleated cells than in BM derived nucleated cells, which practically indicated a higher frequency of MSCs in the nucleated cells of UCB than in those of BM. Secondly, the proliferation analysis revealed that hUCB-MSCs have a faster population doubling time, as compared to BM-MSCs, that

not change after 30 passages. UCB-MSCs also had a higher proliferative capacity of in comparison with BM-MSCs indicating that hUCB-MSCs may be a novel alternative source of human MSCs for clinical applications (Malgieri et al., 2010). Furthermore, Lu et al. (2006) noted the absence of HLA-DR expression on hUCB-MSCs and low expression of HLA-ABC, known as a hurdle for allogenic cell therapies, which may favour the use of hUCB-MSCs for allogenic cell therapy.

In a still growing set of experiments, another population of CB stroma cells was isolated, using the CD45 negative expression and the adherence abilities. These cells were termed unrestricted somatic stem cells (USSCs) (Kogler, 2004, 2006). They were reported to differentiate in vitro into osteoblasts, chondrocytes, adipocytes and neural progenitors as well as in vivo into bone, cartilage, haematopoietic cells and neural and heart tissue (Kim et al., 2005; Kogler et al., 2004). Furthermore, it was even suggested that because USSCs can easily be committed towards the mesenchymal cell lineage, they may represent an early mesodermal progenitor of MSC (Arien-Zakay et al. 2010).

5. *In vitro* differentiation potential of UCSC

5.1 Differential potential into osteogenic, chondrogenic and adipogenic lineages

HUCB-MSCs hold tremendous promise for tissue engineering and regenerative medicine as exposed to appropriate conditions and various factors they can differentiate in vitro along several cell lineages (Figure 4) of three germ layers such as the chondrocyte, osteoblast, adipocyte, myocyte, cardiomyocyte, endothelium, neuronal, astrocyte, oligodendrocyte pancreatic , hepatocyte lineages and others (as further detailed below) (Fan et al., 2011). Several researches reported that certain stimuli direct CB-derived MSCs and USSCs to differentiate into osteoblasts (Jager et al., 2007 as cited in Arien-Zakay et al., 2010; Kogler et al., 2006; Lee et al., 2004 as cited in Arien-Zakay et al., 2010). A recent study comparing the cell-mediated remodeling of three-dimensional collagen I/III gels during osteogenic differentiation of BM-MSCs and hUCB-MSCs showed that both cell types display all features needed for effective bone fracture healing (Schneider et al., 2010). On the contrary, comparing the the chondrogenic potential of hBM-MSCS and hUCB-MSCs it was noted that hUCB-MSC group had 3-times as much collagen as the hBM-MSC group, which supports the former may be a more desirable option for fibro-cartilage tissue engineering (Wang et al., 2009 as cited in Fan et al., 2011). Furthermore, the cell differentiation into chondrocytes was significantly augmented by bone-morphogenetic protein (BMP)-4, a mesodermal factor known to promote chondrogenesis. Additionally, the successful transformation of hUC stroma-derived stem cells into mature adipocytes supports their therapy for esthetic purposes (Karahuseyinoglu et al., 2008 as cited in Fan et al., 2011).

5.2 Differential potential into cardiomyocytes

Attempts to transdifferentiate CB-MSCs towards cardiomyocytes have yielded different results. 5-azacytidine, a chemical analogue of the cytosine nucleoside in the DNA and RNA helix, is currently employed to initiate myogenic differentiation. In spite of the fact that some researches successfully transformed Wharton's jelly derived hUC-MSCs into cardiomyocytes by 5-azacytidine or cardiomyocyte-conditioned medium and noted a slight spontaneous beating (Pereira et al., 2008; Wang et al., 2004 as cited in Fan et al., 2011), others failed to generate cardiomyocyte-like cells from hUC-MSCs, either spontaneously or after treatment with 5-azacytidine (Martin-Rendon et al., 2008). For instance, Roura et al. (2010)

failed to transdifferentiate CB-MSCs towards cardiomyocytes in vitro-the cells did not express cardiomyocyte-specific proteins or presence of calcium rhythmic oscillations or potential-dependent fluorescence emission after various protocols, including addition of chemicals (5-azacytidine and dimethyl sulphoxide), growth factors, Wnt signaling activators and direct contact with neonatal rat cardiomyocytes. Similarly, in other studies there wasn't' any evidence for neocardiomyocyte formation after systemic delivery of CB-mononuclear cells, or direct intramyocardial delivery of CB-133+ cardiac membrane cells, suggesting that there is a limitation in the potential for differentiation of unmodified CB-derived stem cells into cardiomyocytes (Murry et al., 2004). However, MSCs isolated from endothelial/subendothelial layers of the human umbilical cord veins have been proved to have the potential of transdifferentiating into cardiomyocyte-like cells with typical ultrastructure and sarcomers as well as expression of several cardiac-specific genes (Kadivar et al., 2006). Further studies are warranted to reduce the discrepancy between these research papers. However, they suggest that hUC-MSCs can be chemically transformed into cardiomyocytes and considered as a source of cells for cellular cardiomyoplasty.

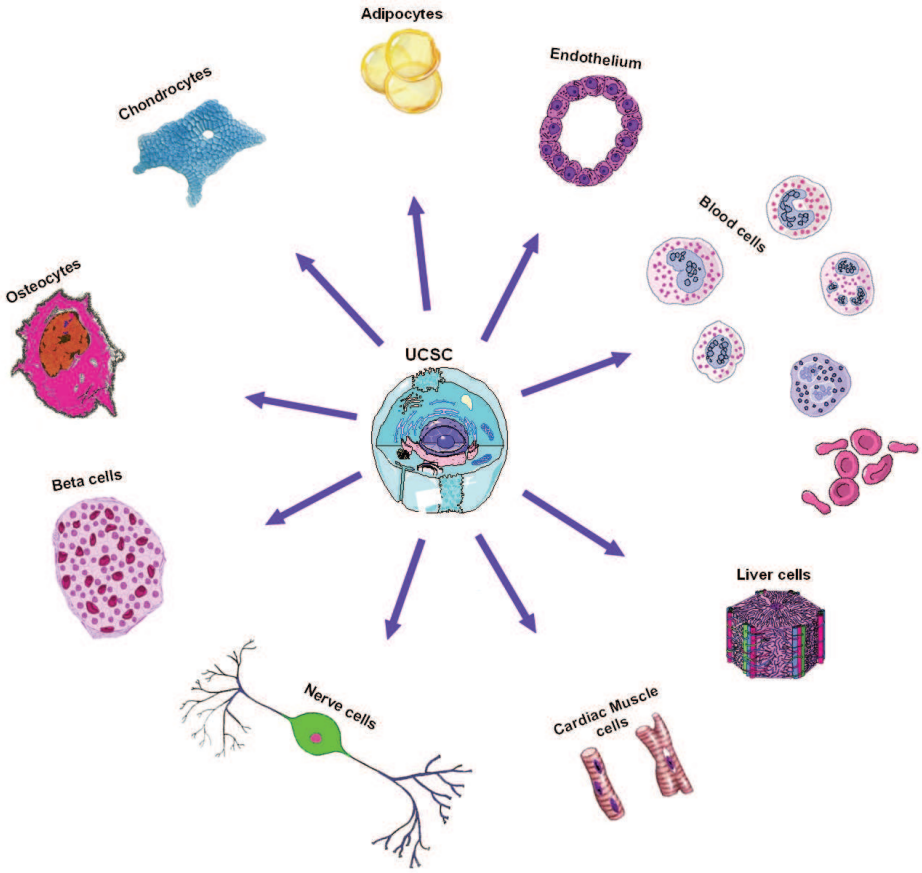


Fig. 4. Umbilical cord mesenchymal stem cell differentiation.

5.3 Differential potential into endothelial cells

Searching for sources with higher availability of endothelial progenitor cells, (Wu et al. 2007) cultured hUC-MSCs in an endothelial differentiation medium containing vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) hUC-MSCs differentiated into endothelial cells as demonstrated by acetylated low-density lipoprotein incorporation and expression of endothelial-specific proteins, such as platelet/endothelial cell adhesion molecule (PECAM) and cd34 (Wu et al., 2007). In comparison of endothelial differentiation of hUC-MSCs and BM-MSCs, some researches noted that hUC-MSCs had higher proliferate potential, higher expression of the endothelial-specific markers after induction and significantly higher total tubule length, diameter and area in angiogenesis assay than those of differentiated BM-MSCs (Chen et al., 2009).

Current studies have addressed the differentiation capacity of CD133 hematopoietic stem cell (HSC), a subset of UCB HSC. In particular, Peichev et al. (2000) have shown that cells isolated from CB expressing both CD34 and VEGFR2 (KDR) also expressed CD133. CD133 was not expressed on mature endothelial cells. The investigators further characterized these cells by illustrating their ability to migrate and differentiate into mature endothelial cells (Peichev et al., 2000). A subsequent study also demonstrated the differentiation of CB-derived CD34+ CD133+ cells into endothelial phenotype in vitro (Pesce et al., 2003).

5.4 Differential potential into neural cells

During the past decade, numerous in vitro studies have demonstrated the generation of neuronal cells from various CB-derived populations. For instance, embryonic stem (ES)-like cell population from CB were found to possess neuroglial progenitor morphology and primitive neuroglia cell markers (Mcguckin et al., 2004). Chen et al. (2005) detected positive expression for antigens typical of brain tissue in other adherent cell subpopulation from CB. CB-derived populations purified by antigen expression were also shown to differentiate into a neural phenotype. CB-CD133 positive cells (Jang et al., 2004 as cited in Arien-Zakay et al., 2010) or CD34− CD45− non-haematopoietic stem cells (Habich et al., 2006 as cited in Arien-Zakay et al., 2010) differentiated into neuronal and glial cells that expressed neuronal and astrocyte-specific markers. Moreover, the full range of neural differentiation ability of the CB-CD45+ multipotential stem cells was demonstrated by the achievement of positive phenotypic and functional markers for dopaminergic neurons, oligodendrocytes and astrocytes (Rogers et al., 2007).

For others, the differentiation potency of hUC-MSCs into neural lineage has attracted extensive attention. The pioneering study in the neuronal induction of hUC-MSCs was accomplished by Mitchell et al. (2004) using a relatively complex and multi-step neuronal induction procedure previously defined (Mitchell et al., 2004; Woodbury et al., 2000 as cited in Fan et al., 2011). In another experiment, hUC-MSCs began to express neuron-specific proteins and exhibit retraction of cell body, elaboration of processes, and clustering of cells after three days' induction with neuronal conditioned medium (NCM). Glutamate invoking inward current was found in the transformed cells between the 9^{th} and 12^{th} days, which suggested that the induced cells differentiated into mature neurons in the post mitosis phase at this stage (Fu et al., 2004). Using a three-step neural induction protocol 60% of the neuroglial cells transformed from hUC-MSCs were stained positive for microtubule-associated protein (MAP-2) and 32% stained positive for GFAP. Moreover, some of them expressed TuJ-1, synaptophysin and γ-aminobutyric acid (GABA). Expression of neuronal markers, such as neuron-specific nuclear protein (NeuN) and MAP2, by induced hUC-MSCs

were also observed by other investigators. More specifically, other investigators observed that induction with protocol similar to Woodbury et al. (2000) resulted in a lower number of cells expressing markers for early neural progenitors (i.e., nestin), however, a greater number of cells expressed a mature neural marker for catecholaminergic cells - tyrosine hydroxylase (TH) (Ding et al. 2007; Kadam et al., 2009; Weiss et al., 2006 as cited in Fan et al., 2011). In addition, Fu et al. (2004) also succeeded in transforming hUC-MSCs into dopaminergic neurons in vitro through stepwise culture in NCM, sonic hedgehog, and FGF-8. These dopaminergic neurons were shown to express the rate-limiting catecholaminergic synthesizing enzyme, tyrosine hydroxylase and to release dopamine into the medium (Fu et al. 2006).

5.5 Differential potential into hepatocyte-like cells

Cirrhosis is a consequence of chronic hepatic injury characterized by replacement of liver tissue by fibrosis and scar tissue, with no effective therapy currently available. Recent developments in stem cell technology have struggled for identifying novel candidate sources of liver cells to be used for regenerative purpose. Instead of ESCs with ethical debates and safety concern, fetal or adult liver cells encumbered by organ availability, BM-MSCs plagued with decreased expansion and differentiation capacities in advanced ages (Roobrouck et al., 2008), Campard et al. (2008) demonstrated that in vitro expanded UC-MSCs constitutively expressed markers of hepatic lineage and genes of enzymes involved in hepatic metabolism after three steps of full term hepatogenic induction. However, absence of some hepatic markers in differentiating UC-MSCs, such as HepPar1 or HNF-4, implied that their differentiation did not reach the level of mature hepatocytes (Campard et al., 2008). A simple one-step induction protocol with hepatocytic growth factor (HGF) and fibroblast growth factor-4 (FGF-4) was also proved to be effective in transforming hUC-MSCs into hepatocyte-like cells expressing the hepatocyte-specific markers albumin (ALB), human α-fetoprotein (AFP) and cytokeratin 18 (CK-18) (Zhang et al., 2009 as cited in Fan et al.,2011). Further research showed that differentiated hUC-MSCs could store glycogen and uptake LDL (Zhang et al., 2009 as cited in Fan et al., 2011). Very recently, UCB derived HSC were also induced to hepatocyte like cells under a 2-step protocol with the combination of the same two growth factors-HGF and FGF-4. Hepatocyte like cells were observed at the end of the protocol (days 14). These differentiated cells were observed to show high expression of genes related to hepatocytes (tryptophan 2, 3-dioxygenase [TO], glucose 6-phosphate [G6P], CK 18, ALB and AFP (Sellamuthu et al., 2011). In addition, the differentiated functional hepatocyte-like cells may still retain their low immunogenicity in vitro (Zhao et al., 2009), which facilitate the allotransplantation to replace the diseased liver cells.

5.6 Differential potential into skeletal muscles

The first successful myogenic transformation of a subset of CD105(+)/CD31(-)/KDR(-) cells from WJ of UC into elongated, multinucleated cells expressing of Myf5 and MyoD in vitro was reported by Conconi et al.(2006).

5.7 Differential potential into Islet-like clusters

In the study by Wang et al. (2010) hMSCs from umbilical cord stroma were induced to differentiate into insulin-producing cells using differentiation medium. Pancreatic beta-cell

development-related genes were expressed in the differentiated insulin-producing cells. Differentiated cells' C-peptide release in vitro increased after glucose challenge (Wang et al., 2010). Another recently published research also showed that WJ- derived hUC-MSCs may serve as a promising alternative cell source of transplantable islet-like clusters (Chao et al., 2008 as cited in Fan et al., 2011). Besides differentiating fresh human UCB into insulin-secreting cells, Phuc et al. (2011) showed that also MSCs derived from banked cord blood (cryopreserved for 1 year) can be differentiated into functional pancreatic islet-like cells in vitro. Having in mind their advantages including large potential donor pool, rapid availability, low risk of rejection and no risk of discomfort for the donor, hUC-MSCs might become an excellent candidate in β-cell replacement therapy of diabetes mellitus.

6. Immune properties of MSCs and in vivo applications

Besides their multi-lineage differentiation potential, UC-derived MSCs have been shown to have immune suppressive action on lymphocyte proliferation through alloantigen and mitogens such as phytohemagglutinin and to reduce the level of proinflammatory cytokines such as interferon-y (IFN-y) and tumor necrosis factor-a (TNF-a). These immune-privileged and immune-modulator properties, recommend them as ideal candidates for cell-based therapies. They fail to induce proliferation of allogeneic lymphocytes in vitro and do not induce an immune response when used in allogenic mismatched animal experimental models (Devine et al., 2003). Furthermore, they have regulatory effects on several cells of the immune system (e.g., T, B, dendritic, and natural killer cells) (DiNicola et al., 2002), prolong skin graft survival (Bartholomew et al., 2002) and have been used in clinical applications to reduce acute and chronic GVHD (Ringden et al., 2006). It has been demonstrated that UCB-MSCs can suppress not only the function of mature dendritic cells but also increase the portion of regulatory T cells (LeBlanc et al., 2004). This regulation of immune response by MSCs is mediated by soluble factors and cell to cell contact mechanisms. Co-culture experiments showed that UCMSCs did not induce any proliferation of resting immune cells and also suppressed the purified T cells or activated human peripheral blood mononuclear cells.

7. In vivo applications of UCSC

7.1 Ischemic vascular disease

7.1.1 Myocardial infarction

In spite of the fact that the treatment of cardiovascular disease and myocardial infarction has benefited over the past 15 years from advances in pharmacologic and intravascular intervention to include placement of drug-eluting stents and interventional re-vascularization, this condition is till associated with significant morbidity and mortality. Furthermore, the fact that there still aren't eligible donors for heart transplant candidates, combined with the limited ability of endogenous cardiomyocytes to divide and repair infarcted myocardium has encouraged the use of stem cell transplantation as adjuvant therapy for cardiovascular disease (Abbott & Giordano, 2003; Ramakrisham et al., 2003 as cited in Goldberg et al., 2007).

Both small clinical trials in humans and animal studies employing cellular therapies have produced data suggesting the efficacious nature of this modality lately. However, as far as patient-derived (adult marrow or mobilized peripheral blood) stem cells are concerned

there are distinct disadvantages for their routine clinical application including the need to subject the patient to stem cell or large volume BM collection at a time of active cardiovascular disease and the needed time for cell culturing for some type of these cells (Goldberg et al., 2007). In addition, there is data suggesting that in patients either at high risk for future cardiovascular disease events or who have documented coronary disease, the body's endogenous stem cell supply and marrow response may be inadequate, as circulating endothelial progenitor cells isolated from patients with increased cardiovascular risk and compared to those isolated from healthy subjects are reduced in number and demonstrate increased degeneration in vitro (Goldberg et al., 2007; Hill et al., 2003).

Additional cellular sources such as embryonic and fetal cardiomyocytes have been studied as a means to improve the failing myocardium (Ramakrisham et al., 2003 as cited in Goldberg et al., 2007). However, besides ethical consideration and possible immune rejection, which are common to both lines, there are other several limitations such as the potential for malignant transformation and for malignant arrhythmias with respect to embryonic stem cells (Abbott & Giordano, 2003; Ramakrisham et al., 2003 as cited in Goldberg et al., 2007) as well as limited availability and sensitivity to ischemia of fetal cardiomyocytes (Etzion et al., 2001; Ramakrisham et al., 2003 as cited in Goldberg et al., 2007) that may preclude their clinical application, encouraging consequent search for other sources of stem cells.

Encouraging results were obtained increasingly from in vivo animal trials using CB stem cells to rejuvenate infracted myocardium, enhance healing and improve ventricular function. It was shown that the human CB mononuclear cells administered into the infracted border progressively improved the function of the left ventricle and reduced the size of infarction in rats with induced acute myocardial infarction (AMI) (Henning et al., 2004). Furthermore, Ma et al., (2005) who experimented with NOD/SCID mice with induced AMI, reported migration of CB mononuclear cells to the heart in 50% of the mice. The size of the infarct zone thus decreased and collagen deposition, as well as increased capillary density were detected, but no myogenesis. Further studies directed towards the ability of UCB CD34+ to improve cardiac function in a model of coronary ischemia were carried out by Hirata et al. (2005). In these experiments, AMI was induced in conditioned male Wistar rats and CB-derived CD34+ cells were injected into the peri-infarct zone. 4 weeks after transplantation, reduction of the left ventricular dilation and improvement of the cardiac function were reported. Moreover, intramyocardial CB cells survived, localized around the vessels of the ischaemic myocardium and capillary density was increased, but only about 1% of the injected cells were incorporated into the myocardium vessels of the rat, indicating that the angiogenic factors released by these cells account for the angiogenesis (Hirata et al., 2005). Similarly, significantly improved cardiac function, markedly increased capillary and arteriole density, and notably decreased apoptotic cells were reported by Wu et al. (2007), who investigated the therapeutic potential of hUC-MSCs in a rat myocardial infarction model. Moreover, it seems that some of transplanted cells managed to live in the infracted myocardium, gathered around arterioles and spread in capillary networks. Noteworthy is the fact that some of them exhibited cardiac troponin-T, von Willebrand factor, and smooth muscle actin, proving that the damaged myocardium regenerated by cardiomyocytic, endothelial, and smooth muscle differentiation of hUC-MSCs in the infracted myocardium (Wu et al., 2007). A specific subset of CB- CD34+ cells, the CD34+ KDR+ cell fraction was suggested as the factor for angiogenesis and improvement of cardiac function for both treatment with mononuclear cells and CD34+ cells (Botta et al., 2004). In a study by Botta et

al. (2004), the myocardium of a NOD/SCID mouse was injected with the CD34+ /KDR+ double selected fraction, isolated from UCB, immediately after the ligation of the left anterior descending artery (LAD). Apoptosis was impaired in this cell population versus CD34+ /KDR- cells in extended culture (up to 22 days). The release of VEGF from the CD34+ /KDR+ cells is thought to provide an autocrine mechanism for survival. The left ventricular end-diastolic pressure decreased as well as the infarct size in the transplanted group for a period up 5 months after AMI induction. The treatment with this cell type also determined more angiogenesis compared with mononuclear cells or CD34+ (Botta et al., 2004). Moreover, immunostaining for human nuclei showed localization of human cells within cardiomyocytes. However, it was not clear whether the transplanted cells had fused, overlapped with or differentiated into cardiomyocytes (Botta et al., 2004; Goldberg et al., 2007). Similar results were reported by Leor et al. (2006), using another immature CB subset, CD133+. CB cells were infused into athymic nude rats with ligated coronary arteries and one month after infusion CB cells had migrated, colonized and survived in the infracted myocardium, being identified near the vessel walls and in the left ventricular cavity. Functionally, the left ventricular fractional shortening improved and the anterior wall thickness diminished (Leor et al., 2006). Ma et al. (2006) reported a comparison of CD133+ expressing cells from UCB and BM in an AMI model. Selected UCB CD133+ cells were injected into injured myocardium of NOD/SCID mice and 4 weeks later the analysis illustrated an approximate 30% absolute mortality reduction in UCB CD133+ -treated animals versus control. Further histological analysis focusing on capillary density produced a 25% increase in this number in cell treated animals versus controls (Ma et al., 2006). In a large animal study, Kim et al. (2005) used a porcine model with chronically occluded LAD and injected CB USSCs directly into the infracted tissue. The grafted cells were detected in the tissue 4 weeks later, the perfusion and wall motion of the infracted area improved whereas the scar thickness decreased (Kim et al., 2005). Overall, these studies suggest a positive effect of a variety of different UCB-derived cell types including cells expressing CD34 and CD133. There are significantly larger numbers of hemangioblasts in UCB which have the same efficacy as that of the BM stem cells. After cell transplantation the ejection fraction and hemodynamics showed cardiac function improvement. It seems that ex vivo long-term cultures do not to affect their ability to increase cardiac function post-infarction (Goldberg et al., 2007).

7.1.2 Buerger's disease

MSCs from CB were also proposed for the treatment of hind limb ischemia. In a pre-clinical study, ischemia was induced in the hind limb of athymic nude mice by femoral artery ligation and human UCB-derived MSCs were transplanted into the ischemic area. Up to 60% of the hind limbs were saved in the femoral artery-ligated animals and the human UCB-derived MSCs were detected by in situ hybridization in the arterial walls of the ischemic hind limb in the treated group (Kim et al., 2006). Buerger's disease, also known as thromboangiitis obliterans, is a nonatherosclerotic, inflammatory, vaso-occlusive disease, characterized as a panangiitis of medium and small blood vessels, including both arteries and adjacent veins, especially of the distal extremities (the feet and the hands). No curative medication or surgery for this disease has been discovered so far. The efficacy of CB-derived MSCs was verified in the treatment of limb ischemia and pain in a clinical case study of Buerger's disease (Kim et al., 2006). HLA-matched human UCB-derived MSCs were transplanted into four men with Buerger's disease who had already failed previous medical

and surgical therapies. As a result, ischemic rest pain suddenly disappeared from their affected extremities, the necrotic skin lesions were healed, the number and size of digital capillaries increased and peripheral circulation improved (Kim et al., 2006), indicating that human UCB-derived MSC transplantation may be a new and useful therapeutic tool for Buerger's disease and similar ischemic diseases.

7.2 Metabolic diseases

7.2.1 Diabetes mellitus

In 2000 there were 150 million people in the world affected by diabetes mellitus (DM) and this number is estimated to double in 2025 (Zimmet et al., 2001 as cited in Fan et al., 2011). Moreover, in the US there are approximately, 15000 youth newly diagnosed with type 1 DM every year (Malgieri et al., 2010). Type 1 DM is the outcome of a cell-mediated autoimmune attack against pancreatic β-cells and the autoimmune response precedes the clinical diagnosis. When hyperglycaemia appears the autoimmune process is significantly advanced due to the fact that 70-80 % of the β-cell mass has been already damaged when the disease started (Couri & Voltarelli, 2008 as cited in Arien-Zakay et al., 2010). Therefore, besides tight control of blood glucose, self blood glucose monitoring and patient education, which have a significant role in the prevention of the development and retard the progression of chronic complication, β-cell mass preservation and/or increase are important targets in management of type 1 DM. Additionally, the replacement of a patient's islets is the only treatment of type 1 DM that achieves an insulin-independent, constant normoglycaemic state and avoids hypoglycaemic episodes (Bretzel et al., 1993 as cited in Fan et al., 2011). Recently, efforts have focused on the use of both autologous and allogenic stem cells as sources of new islets, and perhaps more surprisingly, as potential sources of safe and effective immunomodulation (Limbert et al., 2008). In vitro and in vivo studies have shown that hUCB-derived stem cells and CB-derived MSCs can differentiate into insulin-secreting β-cells (Reddi et al., 2010) expressing pancreatic β-cell markers and synthesizing and secreting functional islet proteins (Gao et al., 2008; Parech et al., 2009 as cited in Arien-Zakay et al., 2010). In the study by Wang et al. (2010) hMSCs from umbilical cord stroma were induced to differentiate into insulin-producing cells using differentiation medium. Pancreatic beta-cell development-related genes were expressed in the differentiated insulin-producing cells. Differentiated cells' C-peptide release in vitro increased after glucose challenge. Further, in vivo glucose tolerance tests showed that blood sugar levels decreased following cells' transplantation into nonobese diabetic (NOD) mice (Wang et al., 2010). Another recently published research also showed that WJ- derived hUC-MSCs may be a promising source of cells of transplantable islet-like clusters (Chao et al., 2008 as cited in Fan et al., 2011). Besides differentiating fresh human UCB into insulin-secreting cells, Phuc et al. (2011) showed that also MSCs derived from banked cord blood (cryopreserved for 1 year) can be differentiated into functional pancreatic islet-like cells in vitro. These results suggest that if human MSCs, especially MSCs from banked cord blood of diabetes patients themselves can be isolated, proliferated, differentiated into functional pancreatic islet-like cells, and transplanted back into them (autologous transplantation), their high-proliferation potency and rejection avoidance will provide one promising therapy for diabetes (Phuc et al., 2011). Recent reports evidenced also that WJ derived-MSCs possess immunomodulatory activities (acting on both innate and acquired immunity effectors) which should reduce the immunogenicity of transplanted cells, thus decreasing rejection. Moreover it has been proposed that MSC administration should be used to alleviate the autoimmune processes

which lead to the destruction of beta cells (Anzalone et al., 2010). Besides reducing blood glucose levels and increasing survival in mouse models of type 1 and type 2 DM (Ende et al., 2004 as cited in Arien-Zakay et al., 2010), CB cell infusion was also proved to improve renal abnormalities and neuropathy caused by DM, suggesting a regenerative action in renal parenchyma and nerves (Ende et al. 2004; Naruse et al., 2005 as cited in Arien-Zakay et al., 2010). Autologous hUCB-MSCs are currently being evaluated in a clinical trial to treat type 1 DM in children (Haller et al., 2008). Moreover, autologous CB infusion in subjects with type 1 DM has been proved not only safe but also efficient by Haller et al. One-year follow-up suggest that autologous CB transfusion is safe and provides some slowing of the loss of endogenous insulin production in the treated children. However, the lack of significant adverse events associated with the study seems to have been the most important result. Nevertheless, we need broader randomized studies as well as 2-year post-infusion follow-up of this cohort to establish whether autologous CB-based approaches can be employed to decrease the endogenous insulin production in children with type 1 DM. This current study is still recruiting children with newly diagnosed type 1 DM, who also have their own CB deposited in a CB bank, for phase II trials. Consequently, the treatment of auto-immune type 1 and 2 DM may greatly benefit from the two positive effects of CB, namely its regenerative abilities and immunomodulatory function (Arien-Zakay et al., 2010).

7.2.2 Hurler syndrome

The mucopolysaccharidoses (MPSs) are inherited metabolic disorders (IMDs) caused by single-gene defects leading to progressive cellular accumulation of glycosaminoglycans (GAGs) and damage to multiple organs, including the central nervous, musculoskeletal or cardiorespiratory systems. Hurler syndrome, the most severe form, is the prototypical model. At present, while available for some conditions, exogenous enzyme replacement therapy is unable to correct cognitive and central nervous system disease because of its inability to cross the blood-brain barrier. In contrast, allogeneic HSC transplantation (HSCT) allows donor-derived, enzyme-producing cells to migrate to the brain and other organs to provide permanent enzyme therapy and thus help somatic organs, improve neurocognitive function and quality of life, and prolong survival, particularly when performed early in the course of the disease. HSCT may also mediate non-hematopoietic cell regeneration or repair. Bone marrow has been the graft source in the past. However, in the last 5 years many patients have been treated with unrelated donor (URD) UCBT, allowing rapid and increased access to transplantation with favorable outcomes (Prasad & Kurtzberg, 2010 a, 2010 b). Sustaining this, CB transplantations led to improved neurocognitive performance and decreased somatic features of Hurler's syndrome (Staba et al., 2004 as cited in Arien-Zakay et al., 2010) and therefore it might be considered as a preferred source of cells for treatment of this disease, as recently suggested after risk factor analysis of patients from the European Group for Blood and Marrow Transplantation (EBMT) (Boelens et al., 2007, 2009].

7.3 Heart valve replacement

Currently used replacements in cardiovascular surgery are made of foreign materials with well known disadvantages such as thrombo-embolic complications, infections, loss of functional and biological properties, and others. (Breymann et al., 2006). Tissue engineering of viable, autologous cardiovascular constructs with the potential to grow, repair, and remodel represents a promising new concept for cardiac surgery, especially for pediatric patients with congenital cardiac defects. Currently, vascular myofibroblast cells (VC)

represent an established cell source for the construction of cardiovascular tissue. Cell isolation requires the invasive harvesting of venous or arterial vessel segments before scaffold seeding, a technique that may not be preferable, particularly in pediatric patients. (Kadner et al., 2002). It has been increasingly shown that, considering their excellent growth properties and tissue formation with biomechanical properties approaching native tissue in vitro, hUC cells seem to represent a promising alternative autologous cell source for cardiovascular tissue engineering, offering the additional benefits of using juvenile cells and avoiding the invasive harvesting of intact vascular structure (Breymann et al., 2006). Hoerstrup et al. (2002) successfully constructed living, autologous pulmonary artery conduits tissue with hUC cells (Hoerstrup et al., 2002 as cited in Fan et al., 2011), while another group published their successful attempt in tissue engineering of autologous human heart valves using cryopreserved vascular umbilical cord cells (Sodian et al., 2006). These results show that establishing autologous human cell banks for pediatric patients diagnosed intrauterinely with congenital defects and likely to need heart valve replacement in the early years of life is of utmost importance (Sodian et al., 2006). Thus, tissue engineering of autologous heart valves with the potential to grow and to remodel is likely to become a fructuous idea. Sustaining these observations, more recently, the same authors described the successful use of cryopreserved umbilical cord blood-derived CD133(+) cells as a single cell source for the tissue engineering of viable human heart valves (Sodian et al., 2006).

7.4 Neurological disorders

7.4.1 Stroke

Ischemic stroke with its immediate neurological injury due to interruption of blood flow to the brain, is a medical emergency of utmost severity: it can trigger severe functional defects in the brain, permanent neurological damage, complications and even death. If it is not quickly diagnosed and treated, it can turn the patient into a huge social and economic burden. The treatment of the stroke has been under constant research by several novel neuron-restorative approaches and it has become the main target for stem cell therapy. In this respect, a variety of stem cells are currently being studied in this research for best implant resource (Fan et al., 2011).

The ability and safety of CB and derived cell populations to protect against neurological deficits was proved in vivo in a variety of injuries models. The first evidence of a therapeutic effect of hUCB came from an experiment where rat was used for middle cerebral artery (MCA) occlusion to induce ischemia. Intravenous administration of hUCB resulted in improved behavioral deficits after stroke in rats (Sanberg et al., 2005). In another recently study the researchers examined the effects of hUCB-MSCs in canine thromboembolic brain model (Chung et al., 2009). Cerebral ischemia was induced through occlusion of the MCA by injecting thrombus emboli into 10 beagles. Infarct volume decreased 1 day after hUCB- MSCs were transplanted through the basilar artery in canine cerebral ischemia whereas infarct volume was increased in the control groups (Chung et al., 2009). Transplanted hUCB-MSCs were differentiated into neurons and astrocytes in and around endothelial cells and expressed neuroprotective factors, such as brain-derived neurotrophic factor (BDNF) and VEGF, at 4 wk transplantation. Additionally, Jeong et al. reported that transplantation of hUCB- MSCs into contralateral regions of injured rat brain at 7 day after injury resulted in significant behavioral improvement and that PKH26-labelled hUCB- MSCs differentiated into neural cells at the injured site 4 wk after transplantation (Jeong et al., 2006 as cited in Kim et al., 2010). Similarly, Chen et al. (2001) demonstrated in a medial carotid artery occlusion model of stroke that upon

CB intravenous administration, many of the physical and behavioral deficits associated with this disease were improved (Chen et al., 2001 as cited in Arien-Zakay et al., 2010). These observations were further supported by other groups, monitoring the dependency of the conferred beneficial effects in the CB cell dose (Vendrame et al., 2004 as cited in Arien-Zakay et al., 2010) and the timing of transplantation after injury (Newcomb et al., 2006 as cited in Arien-Zakay et al., 2010). As mechanisms, the observed beneficial effects afforded by CB therapies were suggested to include reduced inflammation (Vendrame et al., 2006 as cited in Arien-Zakay et al., 2010), apoptotic protection and a combination of trophic actions and nerve fibre reorganization (Xiao et al., 2005 as cited in Arien-Zakay et al., 2010). This observation is suggested to be mediated via the release of growth and repair factors triggered by anoxia (Newman et al., 2006 as cited in Arien-Zakay et al., 2010) as was explored by Arien-Zakay et al. (2009) in vitro, who showed that CB-derived progenitors confer protection towards an insulted neuron by a mechanism involving the release of antioxidants and neurotrophic and angiogenic factors (Arien-Zakay et al., 2009). In another experiment approximately $1*10^6$ clonally expanded hUCB-MSCs were transplanted into the cortex of MCA occlusion rat models. Consequently, significant improved neurological function as well as considerably increased cortical neuronal activity was observed. The transplanted hUCB-MSCs migrated towards the ischemic boundary zone and differentiated into glial, neuronal, doublecortin$^+$, CXCR4$^+$ and vascular endothelial cells. Moreover, hUCB-MSCs transplantation promoted the formation of new vessels to increase local cortical blood flow in the hemispheric and significantly increased expression of neurotrophic factors (Ding et al., 2006 as cited in Fan et al., 2011). It was hypothesized that modulation by stem cell-derived macrophage/microglial interactions and increased β1-integrin expression might enhance the angiogenic architecture as well as plasticity of the ischemic brain after the implantation of hUCB-MSCs (Ding et al., 2006 as cited in Fan et al., 2011). Koh et al. also noted improved neurobehavioral function, reduced infarct volume and increased nestin-positive endogenous stem cells in the hippocampus after transplantation of $6*10^5$ hUCB-MSCs into the damaged hemisphere of immunosuppressed ischemic stroke rats (Koh et al., 2008 as cited in Fan et al., 2011), but only few transplanted MSCs expressed detectable levels of neuron-specific markers. The authors believed that improvement in behavioral function might be connected to the neuroprotective effects of hUCB-MSCs which resulted in increase of endogenous neurogenesis and reduction of infarct volume rather than the formation of new networks between host neurons and the implanted hUCB-MSCs (Koh et al., 2008 as cited in Fan et al., 2011). In agreement with these results, other investigators also observed that transplanted hUCB-MSCs survived for at least 5 weeks in the ischemic brain, significantly reduced injury volume and neurological functional deficits of the subjected rats, widely incorporated into cerebral vasculature with partly differentiation into endothelail cells, and substantially increased vascular density in ipsilateral hemisphere of stroke. It seems that angiogenesis can thus mediate the mechanism for neurological functional recovery after hUCB-MSCs transplantation (Liao et al., 2009 as cited in Fan et al., 2011) and that transplantation of hUCB-MSCs can be useful in clinical trials for ischemia. However, there are serious issues involved, such as the route of transplantation, type of injected cells (hUCB vs hUCB-MSCs) and timing of transplanatation when using stem cell therapy with hUCB-MSCs.

7.4.2 Spinal cord injuries

The consequences of spinal cord injury (SCI), one of the major disabilities which clinical rehabilitation settings face, are: loss of neurons, degeneration of axons, formation of glial scar, and severe functional impairment (Hu et al., 2010). It also involves many negative

factors in that the patients suffer from motor and sensory impairments as well as many other complications during their lifetimes. The restoration of damaged spinal cords has been under many clinical trials during the last two decades but the pharmacological therapies used in clinical settings are few and have limited effects on the regeneration, recovery speed, or retraining of the spinal cord (Hyun & Kim, 2010). Due to their ability to differentiate into neural cell lines replacing non functional tissue, stem cells have attracted the interest of researches. Efforts have been made to establish new synapses and provide a conducive environment. This involved grafting cells from autologous and fetal sources; including embryonic or adult stem cells, Schwann cells, genetically modified fibroblasts, bone stromal cells, and olfactory ensheathing cells and combinations/ variants thereof (Sobani et al., 2010) as well as umbilical cord blood cells (Zhao et al., 2004 as cited in Fan et al., 2011). The potential of transplanted CB and derived populations for the treatment of SCI is under investigation because of their advantages in comparison with other stem cell sources. It was reported that the result of the transplantation of both CB whole fraction and derived CD34 cells was the localization of these cells around the site of injury and improved functional recovery (Nishio et al., 2006; Saporta et al., 2003 as cited in Arien-Zakay et al., 2010). Furthermore, the transplantation of hCB in a spinal cord-injured female patient was associated with improved sensory perception and mobility, both morphologically and functionally and regeneration of the spinal cord at the injured site and some of the cauda equine below it, as shown by computed tomography (CT) and magnetic resonance imaging (MRI) (Kang et al., 2005 as cited in Arien-Zakay et al., 2010). Kuh et al. studied the effects of transplanted HUCBs ± BDNF on moderate degree spinal cord injured rats. The HUCBs transplanted group improved, more than the control group at every week after transplantation, and also, the BDNF enabled an improvement of the Basso, Beattie and Bresnahan (BBB) locomotion scores since the 1 week after its application. 8 weeks after transplantation, the HUCBs with BDNF transplanted group had more greatly improved BBB scores, than the other groups. Additionally, the transplanted HUCBs were differentiated into various neural cells. The HUCBs and BDNF each have individual positive effects on axonal regeneration. The HUCBs can differentiate into neural cells and induce motor function improvement in the cord injured rat models. Especially, the BDNF has effectiveness for neurological function improvement due to axonal regeneration in the early cord injury stage. The authors concluded that the HUCBs and BDNF have recovery effects of a moderate degree for cord injured rats (Kuh et al., 2005). Phase I/II clinical trials are in progress in the China Spinal Cord Injury Network (http://www.clinicaltrials.gov), aiming to determine the feasibility, safety, efficacy and optimal dose of CB mononuclear cell transplantation as a method to treat patients with chronic SCI (Arien-Zakay et al., 2010). Due to their high ability to differentiate into nerve like cells hUCB-MSCs hold great promise as tools for understanding development and as therapeutic agents for spinal cord injury. Transplantation of hUCB-MSCs into the injured spinal cord may have the following functions-: compensation for demyelination; removal of inhibition; promotion of axonal regeneration; direction of axons to appropriate targets and lost cells replacement (Malgieri et al., 2010). A promising research reported by Yang et al. (Yang et al., 2008 as cited in Fan et al., 2011) provided evidence that transplantation of WJ-derived hUC-MSCs was an effective strategy to promote the regeneration of corticospinal fibers and locomotor recovery after spinal cord transaction in the rat. The results were as follows: several weeks after transplantation of hUC-MSCs, significant improvements in locomotion and fewer astrocytes in the lesion site, activated microglia in rostral and caudal stumps of the lesion as well as

increased numbers of regenerated axons in the corticospinal tract and neurofilament – positive fibers around the lesion site were observed as compared with the control group (Yang et al., 2008 as cited in Fan et al., 2011). Moreover, transplanted hUC-MSCs survived as long as 16 weeks, migrated from the implantation site for about 1.5 mm in the caudal direction of the rostrocaudal axis and produced large amounts of human neutrophil-activating protein-2, neurotrophin-3 (NT-3), bFGF and VEGF receptor 3 in the host spinal cord, which may help spinal cord repair (Yang et al., 2008 as cited in Fan et al., 2011). The regeneration of several corticospinal axons after transplantation of hUC-MSCs is likely to be sustained by the release of more cytokines or growth factors from the undifferentiated stem cells rather than differentiation of these cells into neuronal or glial cells. The authors also believe that the implanted hUC-MSCs are able to modulate the activities of microglia and reactive astrocytes (Yang et al., 2008 as cited in Fan et al., 2011). In tight connection with this, Zhang et al. (2009) noted that in the case of WJ cells-derived neurospheres transplanted in combination with BDNF into transected spinal cord rats, the number of survivor grafted cells was very small while BBB scores and axonal regeneration significantly increased and reduced cavitations were noticed. These results might be explained through the axonal regeneration and neuroprotective action activated by the grafted cells (Zhang et al., 2009 as cited in Fan et al., 2011). In another recent study, the treatment with hUC-MSCs proved to facilitate functional recovery of hindlimb locomotor function after traumatic spinal cord injury in rats, at 5 wks after transplantation. This recovery was accompanied by increased length of neurofilament-positive fibers and increased numbers of growth cone-like structures around the lesion site. Transplanted hUC-MSCs survived, migrated over short distances, and produced large amounts of glial cell line-derived neurotrophic factor and neurotrophin-3 in the host spinal cord. There were fewer reactive astrocytes in both the rostral and caudal stumps of the spinal cord in the hUC-MSCs group than in the control group (Hu et al., 2010). Mesenchymal stem cells can also differentiate into Schwann-like cells. Yan-Wu et al. (2011) induced hUC-MSCs in vitro into neurospheres constituted by neural stem-like cells, and further into cells bearing strong morphological, phenotypic and functional resemblances with Schwann-like cells. These HUMSC-derived Schwann-like cells, after grafting into the injured area of the rats' SCI, had a partial therapeutic effect in terms of improving the motor function. Furthermore, significant improvement in rats treated by Schwann-like cell grafting combined with NT-3 administration was demonstrated in the behavioral test as compared with that in animal models received the cell grafting only, suggesting that this combined administration may represent a new strategy of stem cell therapy for spinal cord injury (Yan-Wu et al., 2011). Taking into account these encouraging pre-clinical and clinical trials, reporting improvement of neurological deficits together with the increasing amount of data on the differentiation of CB-populations into a variety of neuronal phenotypes it may be assumed that CB will become an important factor for treatment of neurological illnesses.

7.4.3 Parkinson's disease

Several studies also investigated the CB therapeutic potential in neurodegenerative diseases, including Parkinson's disease (PD), Alzheimer's disease and amyotrophic lateral sclerosis. PD is a neurodegenerative disorder characterized by the progressive loss of striatal dopaminergic function. To date, a variety of stem cells have been explored to find a promising therapy for this unsatisfactorily treated disease in clinical settings (Yasuhara & Date, 2007 as cited in Fan et al., 2011). Fu et al. (2006) showed that in vitro hUC-derived

MSCs transformed into dopaminergic neurons expressing the rate-limiting catecholaminergic synthesizing enzyme, thyrosine hydroxylase and releasing dopamine into the medium (Fu et al., 2006). Transplantation of these dopaminergic neurons into the striatum of Parkinsonian rats could partially relieve the lesion-induced amphetamine-evoked rotation, indicating an amelioration of motor deficits. Similar results were demonstrated by Weiss et al. (2006), who also treated rat models affected by PD with hUC-MSCs. The transplantation of approximately 1,000 undifferentiated hUC-MSCs into striatum of hemiparkinsonian rats without immune suppression produced an amelioration in apomorphine-induced rotations in the pilot test without evidence of formation of brain tumors as well as a frank host immune rejection response. The hUC-MSCs produced significant amounts of glial cell line-derived neurotrophic factor (GDNF), one of the most potent trophic factors for dopaminergic neurons and FGF (Weiss et al., 2006 as cited in Arien-Zakay et al., 2010). More importantly, the TH-positive DA neurons in the substantia nigra (SN) and ventral tegmental area (VTA) showed a valid correlation between the number of cells and the number of apomorphine-induced rotations. Therefore, the behavioral recovery of PD model animals may contribute to rescue of the degenerating DA neurons in the SN and VTA (Weiss et al., 2006 as cited in Arien-Zakay et al., 2010). It appears that Li et al. (2010) are the first who described the induction of human umbilical vein mesenchymal stem cells (HUVMSCs) into dopaminergic-like cells. In their study, HUVMSCs were induced in vitro into neurospheres constituted by neural stem-like cells, and further into cells bearing strong morphological, phenotypic and functional resemblances with dopaminergic-like cells. These HUVMSC-derived dopaminergic-like cells, after grafting into the brain of a rat model of PD, showed a partial therapeutic effect in terms of the behavioral improvement. Moreover, nerve growth factor (NGF) administration significantly promoted the survival of the grafted cells in the host brain and enhanced the content of dopaminergic in the local brain tissue and behavioral test demonstrated a significant improvement of the motor function of the PD rats after dopaminergic-like cell grafting with NGF administration as compared with that of rats receiving the cell grafting only, which might indicate this combination as a good and new strategy of stem cell therapy for PD (Li et al., 2010).

7.4.4 Amyotrophic Lateral Sclerosis (ALS)

Stem-cell transplantation is an attractive strategy for neurological diseases and early successes in animal models of neurodegnerative disease generated optimism about restoring function or delaying degeneration in human beings (Silani et al., 2004). Considering the lack of effective drug treatments for amyotrophic lateral sclerosis (ALS), and compelling preclinical data, stem-cell research has highlighted this disease as a candidate for stem-cell treatment. In a mouse model of ALS, CB cells transplantation resulted with delayed disease progression and improved survival of diseased mice, while the transplanted cells survived 10-12 weeks after infusion entering regions of motor neuron degeneration in the brain and spinal cord (Garbuzova-Davis et al., 2003 as cited in Arien-Zakay et al., 2010), as monitored according to transplanted cell dose (Garbuzova-Davis et al., 2008 as cited in Arien-Zakay et al., 2010). ALS may also benefit from combined gene/stem-cell approaches. In the study by Rizvanov et al. (2011), mononuclear fraction of hUCBCs were transfected by electroporation with dual plasmid constructs, simultaneously expressing VEGF(165) and human fibroblast growth factor 2 (FGF(2)) (pBud-VEGF-FGF(2)). These genetically modified hUCBCs were injected retro-orbitally into presymptomatic ALS transgenic animal models ((G)93(A) mice).

The report results demonstrated that genetically naïve hUCBCs may differentiate into endothelial (CD34+) and microglial (iba1+) cells; however when over-expressing VEGF-FGF(2), hUCBCs transform into astrocytes (S100+). Autocrine regulation of VEGF and FGF(2) on hUCBCs, signal molecules from dying motor neurons in spinal cord, as well as self-differentiating potential may provide a unique microenvironment for the transformation of hUCBCs into astrocytes that eventually serve as a source of growth factors to increase the survive potential of surrounding cells in the diseased regions (Rizvanov et al., 2011).

7.4.5 Alzheimer's disease

Alzheimer's disease (AD) is a proeminent neurodegenerative disease, marked clinically by insidious dementia. The neuropathological hallmarks of AD include the presence of extracellular amyloid-beta peptide (Abeta) in the form of amyloid plaques in the brain parenchyma and neuronal loss. The mechanism associated with neuronal death by amyloid plaques is unclear but oxidative stress, glial activation and inflammatory changes have been implicated (Lee et al., 2010a, 2010b). The umbilical cord blood stem cells have been studied also in AD and they were shown to markedly diminish the beta-amyloid plaques and associated astrocytosis in an AD mouse model (Nikolic et al., 2008 as cited in Arien-Zakay et al., 2010). In addition, two very recent studies have demonstrated that hUCB-MSCs can be an efficient potential therapeutic agent in AD. Therefore, transplanted hUCB-MSC into amyloid precursor protein (APP) and presenilin1 (PS1) double-transgenic mice significantly improved spatial learning and memory decline and dramatically reduced amyloid-beta peptide (Abeta) deposition, beta-secretase 1 (BACE-1) levels and tau hyperphosphorylation. Interestingly, these effects were associated with reversal of disease-associated microglial neuroinflammation, as evidenced by decreased microglia-induced proinflammatory cytokines, elevated alternatively activated microglia, and increased anti-inflammatory cytokines. These results lead us to suggest that hUCB-MSC produced their sustained neuroprotective effect by inducing a feed-forward loop involving alternative activation of microglial neuroinflammation, thereby ameliorating disease pathophysiology and reversing the cognitive decline associated with Abeta deposition in AD mice (Lee et al., 2010 a). Moreover, the same authors examined in vitro the potential impact of hUCB-MSCs treatment on neuronal loss using a paradigm of cultured hippocampal neurons treated with Abeta, confirming that hUCB-MSCs co-culture reduced the hippocampal apoptosis induced by Abeta treatment. Furthermore, in an acute AD mouse model to directly test the efficacy of hUCB-MSCs treatment on AD-related cognitive and neuropathological outcomes, they shown that markers of glial activation, oxidative stress and apoptosis levels were decreased in AD mouse brain. Interestingly, hUCB-MSCs treated AD mice demonstrated cognitive rescue with restoration of learning/memory function (Lee et al., 2010 b).

7.4.6 Infantile cerebral paresis and other cerebral impairments

Traumatic brain injury is also a potential target for treatment using CB cells as it was first described by Lu et al. (2002) in a rat model. The transplanted cells migrated into the site of brain lesion and a decrease of neurological damage was documented (Lu et al. 2002 as cited in Arien-Zakay et al., 2010). Moreover, brain damage around birth may trigger lifelong neurodevelopmental deficits and cerebral palsy is estimated to affect 10 000 infants annually. Brain damage of the hypoxic-ischemic kind around birth is also treatable with CB cells. Using brain-damaged neonatal rats, Meyer et al. (2006) observed that the neurological

effects of cerebral palsy were corrected- spastic paresis was largely alleviated and CB mononuclear cells were incorporated in the lesion brain area without obvious signs of transdifferentiation (Meyer et al., 2006 as cited in Arien-Zakay et al., 2010). The feasibility of CB collection, preparation and autologous infusion in babies born with signs of brain injury is now being tested in a pilot study conducted by Duke University (http://www.clinicaltrials.gov) (Arien-Zakay et al., 2010).

7.5 Hemoglobinopathies. Fanconi anemia

Allogeneic stem cell transplantation is the only curative option for patients with hereditary bone marrow failure syndromes. But because nearly two-thirds of patients requiring HSCT will not have a suitable related donor, the applicability of HSCT to larger numbers of patients has been augmented with the increasing availability of unrelated donors. Presently, alternative HSC sources include unrelated donor (URD) bone marrow (BM) or peripheral blood stem cells (PBSC) and unrelated donor umbilical cord blood (UCB). While URD BM and PBSC transplants have a proven track record of success, the search process takes 3-4 months, which is often longer than patients with high risk disease can wait. Despite nearly 13 million registered volunteer donors worldwide, nearly half of patients still do not have a closely HLA-matched donor. The applicability of HSCT markedly expanded with the introduction of UCBT, particularly for racial and ethnic minorities (Smith & Wagner, 2009). The first related donor UCBT was performed in 1988 (Gluckman et al., 1989 as cited in Barker & Wagner, 2003) and the first URD UCBT in 1993. Since those first reports, it has become clear that UCB is a safe and effective source of HSC for transplant. Moreover, the demonstration that hematopoiesis could be reliably reconstituted after myeloablative conditioning prompted the development of repositories of banked, HLA-typed UCB. With approximately 350,000 units banked worldwide (http://www.bmdw.org), the addition of UCB to the available stem cell sources makes it possible for nearly everyone who requires an HSCT to have a suitable donor available (Smith & Wagner, 2009). Sustaining this, a very recent multicenter, retrospective study, based on data reported to the Eurocord Registry about patients with hereditary bone marrow failure syndrome (other than Fanconi anemia) who underwent umbilical cord blood transplantation showed that related UCBT is associated with excellent outcomes while increasing cell dose and better HLA matching might provide better results in unrelated UCBT (Bizzeto et al., 2011).

Fanconi anemia (FA) is a rare autosomal recessive disease characterized by excessive chromosomal breakage, congenital abnormalities, progressive bone marrow failure and a predisposition to leukemia and epithelial malignancies (Smith AR & Wagner JE, 2009). The hematopoietic stem cell transplantation is the only curative treatment of this disease (Bielorai et al., 2004).

The first CB transplant was performed in 1988 in a patient with Fanconi anemia. The donor was his HLA-identical sister who was known by pre-natal diagnosis to be HLA identical and not affected by the Fanconi mutation. To date, there have been no formal comparisons between the alternative donor sources. As with hemoglobinopathies, URD transplant remains the gold standard until retrospective or prospective comparative trials can be performed. However, for patients who do not have an HLA-matched donor or who cannot wait the time it takes to complete a donor search, UCB is a reasonable alternative (Smith & Wagner, 2009).

More comparative studies are needed before definitive conclusions can be made in nonmalignant diseases, but the available data indicate UCB as a feasible alternative HSC

source in most patient populations. In general, though, 8/8 HLA-matched BM remains the 'gold standard' for alternative donor HSCT, but UCB should be considered a reasonable option in those that do not have such a donor available and for those in whom the time to transplant is critical, such that waiting for an URD BM would not be in the best interest of the patient. Current practice has been to select the UCB unit that is 0-2 HLA antigen-mismatched with the patient with the highest cell dose. Further efforts focused on increasing the number, HLA diversity and quality of stored UCB units as well as addressing cell dose limitations using strategies, such as double UCB transplant and ex vivo expansion of a single unit are needed to continue to advance the field of UCBT (Barker & Wagner, 2003; Smith &Wagner, 2009).

7.6 Hematological malignancies

Hematological malignancy is the most common indication for allogeneic HSCT in both children and adults. The choice of HSC source depends on both patient and disease characteristics and the urgency of the transplant. Because of the rapid availability of units, UCB is a particularly attractive option (Smith & Wagner, 2009).

7.6.1 In children

Infant leukemia is a particularly challenging form of leukemia to treat and the decision of whether to treat with intensive chemotherapy or to proceed with URD transplant when a suitable related donor is not available is a difficult one.

On behalf of the Center for International Blood and Marrow Research (CIBMTR), Eapen et al. (2007) recently reported the outcomes of 785 children with acute leukemia comparing outcomes in recipients of UCB (n=503) and URD BM (n=282). The most notable finding was that UCB compared favorably to the 'gold standard' of 8/8 allele-matched unrelated BM. In fact, the 5-year leukemia-free survival (LFS) was similar after 8/8 matched unrelated BM (MUBM), mismatched unrelated bone marrow (MMUBM) and mismatched UCB (MMUCB) with higher survival in recipients of matched UCB (MUCB). The incidence of acute and chronic GVHD was similar between the groups. While treatment-related mortality (TRM) was higher after two-antigen MMUCB, a lower risk of relapse resulted in comparable survival outcomes for this cohort. This research was unique in that UCB was compared to the present day standard of allele-level HLA-matched BM donors. These findings support the use of HLA-matched or -mismatched UCB in children with high risk acute leukemia who need transplantation. (Eapen et al 2007 as cited in Smith &Wagner, 2009).

7.6.2 In adults

In contrast to the outcomes in children, HSCT in adults is typically associated with higher risks of GVHD, infections, delayed immune reconstitution and increased TRM, partly related to a higher likelihood of comorbidities at the time of transplant. In contrast to children, use of UCB in adults has been more restricted due to cell dose limitations. The safety and feasibility of UCBT in adults with hematological malignancies was first reported in 2004 (Laughlin et al., 2004; Rocha et al., 2004 as cited in Smith &Wagner, 2009).

The outcomes in 1240 adults (148 UCB, 243 MUBM, 111 MMUBM, 518 matched PBSC MPBSC and 210 mismatched PBSC MMPBSC) were examined and the results were published recently in an abstract on behalf of the CIBMTR (Eapen et al., 2008 as cited in Smith &Wagner, 2009). Contrary to the previous data (Laughlin et al., 2004; Rocha et al, 2004

as cited in Smith & Wagner, 2009), all unrelated donor BM and PBSC grafts were matched at allele level for HLA-A, -B, -C, and –DRB1. In this analysis, TRM was lower and LFS higher when MUBM and MPBSC were used as compared to the other sources, suggesting that these graft sources are preferred when available and time permits. However, partially HLA-matched UCB with an adequate cell dose (≥ 2.5×10^7 nucleated cells/kg) is a suitable alternative when an HLA-matched URD is not available or when the transplant is urgent (Eapen et al., 2008 as cited in Smith &Wagner, 2009).

Analyzing a more genetically heterogeneous group of patients, Kumar et al. (2008) also showed superior outcomes in UCB recipients relative to those transplanted with other sources of HSC. Patients receiving UCB had the lowest TRM and highest 3-year LFS (61% vs. 27%, 13% and 14% in the matched related donor, matched unrelated donor (MURD) and mismatched unrelated donor (MMURD) groups, respectively). Taken together, these results at least advocate for continued investigations into the use of UCB as an alternative stem cell source for the treatment of adults with hematological malignancy (Kumar et al., 2008 as cited in Smith & Wagner, 2009).

In summary, these retrospective studies suggest for children, the first line HSC source would be a 6/6 MUCB provided that the cell dose is adequate. The probability of finding a 6/6 MUCB, however, is low (~10%). However, results in recipients of 8/8 MUBM and 5/6 MMUCB and 4/6 MMUCB are similar, suggesting that any of these options are reasonable. In this case, the decision must be individualized and based on the urgency of the transplant and potential need for future donor lymphocyte infusion (DLI). However, it is clear that with UCB, TRM increases with each degree of HLA-mismatch, so higher cell doses are needed with more HLA disparity. In addition, PBSC or BM is often a more realistic HSC option in adults due to cell dose limitations. Therefore, in adults, cell dose limitations with UCB units give the advantage to HLA matched BM and PBSC. For all patients, if an 8/8 MUBM is not available, no one source stands out. UCB has the advantage of rapid availability while BM and PBSC have the advantage of availability of DLI (Smith & Wagner, 2009).

7.7 Liver diseases

Liver cell transplantation is a promising technique for the treatment of metabolic liver disease, liver fibrosis and other end stage liver diseases. In vivo investigations showed that in carbon tetrachloride (CCl4)-induced cirrhosis in a rat model, hUCB-MSCs infusion demonstrated inhibition of TGF-β, collagen type I and α-smooth –muscle-actin (α-SMA) expression as well as expression of hepatocyte-specific markers human ALB and AFP in injured liver (Jung et al., 2009), which indicated they might become an alternative source for liver-directed cell therapies (Campard et al., 2008). Several weeks after transplantation into the lesion livers of CCl4-induced liver fibrosis rats, significant reduction in liver fibrosis with lower levels of serum glutamic oxaloacetic transaminase, glutamic pyruvate transaminase, α-SMA and TGF-β in the liver was observed. Moreover, up-regulated expression of hepatic mesenchymal epithelial transition factor-phosphorylated type (Met-P) and hepatocyte growth factor was also found (Tsai et al., 2009 as cited in Fan et al., 2011). The engrafted hUCB-MSCs did not differentiate into hepatocytes expressing human ALB or AFP but secrete a variety of bioactive cytokines, including human cutaneous T cell-attracting chemokine, leukemia inhibitory factor, and prolactin, which may benefit the restoration of liver function and promotion of regeneration (Tsai et al., 2009 as cited in Fan et al., 2011). Similar data were noted by Yan et al. (2009). In similar CCl-4 –injured mouse

hepatic injury, hUCB-MSCs were discovered to express tryptophan 2, 3-dioxygenase, human AFP, CK18, α-SMA after administration into the damaged livers. Transplanted hUCB-MSCs could reduce hepatocyte denaturation, inhibit hepatocyte apoptosis, diminish serum aminotransferases and facilitate hepatocyte proliferation (Yan et al., 2009 as cited in Fan et al., 2011).

Lin et al. (2010) tested the use of WJ stem cells (WJSCs) in treating chemically induced liver fibrosis via intraperitoneal injection of thioacetamide. They noticed that transplanted cells were distributed in the fibrotic area and around blood vessels, and hepatic recovery was accelerated. Serum prothrombin time significantly recovered, and serum albumin also improved at 21 days posttransplantation whereas collagen accumulation also decreased at 14 days. Thus, human WJSCs promoted recovery after chronic liver damage. Moreover, transplanted WJSCs produced albumin, hepatocyte growth factor (HGF), and metalloproteinase (MMP) after transplantation to chemically injured liver, indicating that WJSC may help to decrease liver collagen and thus may be useful for treating liver fibrosis (Lin et al., 2010). According to all these results, hUCB-MSCs could be useful in liver therapy. It should not be forgotten that liver contains endogenous abundant progenitor cells for recovery of liver damage. Therefore, it is currently difficult to determine which stem /progenitor cell populations are best for liver disease therapy (Kim et al., 2010).

7.8 Lung diseases

Progression of acute respiratory distress syndrome is documented by loss of lung tissue as a result of inflammation and fibrosis. The role of term human UC cells derived from WJ with a phenotype consistent with MSCs was tested in the treatment of a bleomycin-induced mouse model of lung injury. Injected MSCs were located in the lung 2 weeks later only in areas of inflammation and fibrosis but not in healthy lung tissue. The administration of MSCs decreased inflammation, inhibited the expression of TGF-beta, IFN-gamma, and the proinflammatory cytokines macrophage migratory inhibitory factor and TNF-α and reduced collagen concentration in the lung. MSCs also increased matrix metalloproteinase-2 levels and reduced their endogenous inhibitors, tissue inhibitors of matrix metalloproteinases, favoring a pro-degradative milieu following collagen deposition. Notably, injected human lung fibroblasts did not influence either collagen or matrix metalloproteinase levels in the lung. The results of this study suggest that MSCs participate in anti-fibrosis in lung injury and may increase lung repair if used to treat acute respiratory distress syndrome (Moodley et al., 2009). Others examined whether intratracheal or intraperitoneal transplantation of human UCB-derived MSCs can attenuate hyperoxia-induced lung injury in immunocompetent newborn rats. Wild-type rats were randomly exposed to 95% oxygen or air from birth and in the transplantation groups, a single dose of PKH26-labeled human UCB-derived MSCs was administered either intratracheally (2×10^6 cells) or intraperitoneally (5×10^5) cells) at postnatal day 5. The harvested lungs were examined after 10 days and two abilities of hUCB-MSCs, immune modulation and differentiation potential towards the respiratory epithelium were assessed. Despite one fourth dosage of MSCs, significantly more PKH26-labeled donor cells were recovered with intratracheal administration than with intraperitoneal administration both during normoxia and hyperoxia. The hyperoxia-induced impaired alveolarization and increased level of TNF-alpha and TGF-beta mRNA, alpha-SMA protein, and collagen were significantly alleviated only with intratracheal MSCs transplantation whereas the hyperoxia-induced increase in the number of TUNEL-positive cells, myeloperoixdase activity, and the level of IL-6 mRNA

were significantly attenuated with both intratracheal and intraperitoneal MSCs transplantation. As MSCs differentiated into respiratory epithelium in vitro and a few PKH26-positive donor cells were colocalized with pro surfactant protein C in the damaged lungs, hUCB-MSCs could be employed in cell therapy via both anti-inflammation and regeneration in hypoxia induced lung injury, representing a possible candidate for the new therapeutic modality for the hyperoxia-induced neonatal lung diseases, such as clinical bronchopulmonary dysplasia (Chang et al., 2009; Malgieri et al., 2010).

7.9 Cartilage regeneration

Mature articular cartilage is vulnerable to injuries and disease processes that cause irreversible tissue damage because of its limited capacity for self-repair. UCB is a source of MSCs which can give rise to cells of different lineages, including cartilage or bone (Mara et al., 2010). In fact hUCB-MSCs have much higher chondrogenic differentiation potentials which might lead to regeneration of damaged cartilage. In addition to this chondrogenic differentiation potential of MSCs, recent advances in our understanding of the regeneration mechanism for cartilage defects have demonstrated that MSCs also show potent immunosuppression and anti-inflammatory effects (Di Nicola et al., 2002; Hao et al., 2009, Oh et al., 2008, Wang et al., 2009 as cited in Kim et al., 2010). These properties might be due in part to some specific secreted cytokines and growth factors, suggesting that chondrogenic differentiation and paracrine actions might be involved in replacement of damaged cartilage tissues and stimulation of the regeneration process (Kim et al., 2010). Wang et al. (2009) conducted for the first time a 6-week study comparing the differentiation potential of hBMSCs and hUCMSCs towards condrocytes in a three-dimensional (3D) scaffold. They concluded that hUCMSCs may be a desirable option for use as a mesenchymal cell source for fibrocartilage tissue engineering, based on abundant type I collagen and aggrecan production of hUCMSCs in a 3D matrix. Anyway, the authors stated that further investigation are warranted in order to find the signals that best promote also type II collagen production of hUCMSCs for hyaline cartilage engineering (Wang et al., 2009). Similar, a very recent study demonstrated that functionally, CB-MSC could be more readily induced to differentiate into chondrocytes than could BM-MSC and adipose tissue-MSC (AT-MSC). Moreover, CB-MSC showed immunosuppressive activity equal to that of BM-MSC and AT-MSC, indicating CB a practical source of MSC for cell therapy and regenerative medicine through the use of the well established CB banking system (Zhang et al., 2011). Another attempt to differentiate cells from UCB into chondrocytes with insulin-like growth factor 1 (IGF-1) and transforming growth factor-ss3 (TGF-ss3) showed that TGF-ss3 used in micromass culture is the best growth factor for promoting the proliferation and differentiation of mesenchymal cells from UCB during chondrogenesis. The western blot analysis revealed that after 3 weeks, the expression of type II collagen was greater in micromass culture with TGF-ss3 (Mara et al., 2010).

Two representative common diseases of cartilage degeneration include osteoarthritis and rheumatoid arthritis. Rheumatoid arthritis (RA) is a T-cell-mediated systemic autoimmune disease that primarily attacks synovial joints, leading to articular destruction and functional disability. The study by Liu et al. (2010) is the first report of the UC-MSCs use in the treatment of RA. The potential immunosuppressive effects of human UC-MSCs in RA were evaluated, too. Both the actions of UC-MSCs on the responses of fibroblast-like synoviocytes (FLSs) and T cells in RA patients and the possible molecular mechanism mediating this immunosuppressive effect of UC-MSCs and the therapeutic effects of systemic infusion of

human UC-MSCs on collagen-induced arthritis (CIA) in a mouse model were explored. They demonstrated that UC-MSCs exerted a profound inhibitory effect on the proliferation, invasive behavior and inflammatory responses of FLSs, suppressed T cell activation in vitro and induced the generation of regular T cells (Tregs). More importantly, in mice, systemic infusion of UC-MSCs significantly reduced the severity of CIA, strongly suggesting that UC-MSCs might be a therapeutic strategy in RA. In addition, the immunosuppressive activitiy of UC-MSCs could be prolonged by the participation of Tregs (Liu et al., 2010). Despite growing evidence, the molecular mechanisms underlying cartilage repair and regeneration by hUCB-MSCs remain not settled. Therefore, extensive studies of hUCB-MSCs therapeutic mechanisms are required for an understanding of their regenerative potential and for efficient and safe clinical application. Supported by the clinical trial phase I/II results (NCT01041001), adult stem cell therapy using hUCB-MSCs for cartilage degenerative disease might be a promising alternative to previous treatments (Kim et al., 2010).

7.10 Gene therapy

Retroviral mediated gene transfer has been shown to be an efficient method for introducing genetic sequences into mammalian cells (Bernstein et al., 1985; Friedmann et al., 1989 as cited in Lu et al., 1996) and gene replacement therapy has been proposed for many single gene disorders (Lu et al., 1996). Adeno-associated viruses (AAV) can also be used as a vehicle for gene transfer. MSCs are amenable to be genetically modified, which makes them become a promising platform for cell and gene therapy and broadens their potential therapeutic applications in several fields, including improvement engraftment following stem cell transplantation and acceleration of hematopoietic reconstitution, treatment of severe graft-versus-host disease, utilization in targeting tumors and delivering anti-cancer molecules as well as cellular vehicle for protein-supplement gene therapy (Fritz & Jorgensen, 2008; Kumar et al., 2008 as cited in Fan et al., 2011). The strategy that uses therapeutic gene-transfected hUCMSCs as cellular vehicles for targeted biologic agent delivery has solved the problem of short half-life or excessive toxicity of biological agent(s) in vivo. Administration of hUC-MSCs expressing interferon-β with (Rachakatla et al., 2008 as cited in Fan et al., 2011) or without 5-fluorouracil (Rachakatla et al., 2007 as cited in Fan et al., 2011) was found to target to experimentally developed lung tumors and significantly reduce the tumor burden. Furthermore, the co-culture of a small numbe of IFN-beta gene-transfected hUCMSCs (IFN-beta-hUCMSCs) with the human bronchioloalveolar carcinoma cell lines H358 or SW1573 significantly inhibited growth of both types of carcinoma cell lines, whereas systemic administration of IFN-beta-hUCMSCs markedly attenuated growth of orthotopic H358 bronchioloalveolar carcinoma xenografts in SCID mice by increasing apoptosis. These results clearly indicate that IFN-beta-hUCMSCs caused cell death of bronchioloalveolar carcinoma cells through IFN-beta production, thereby attenuating tumor growth in vivo, suggesting that IFN-beta-hUCMSCs are a powerful anti-cancer cytotherapeutic tool for bronchioloalveolar carcinoma (Matsuzuka et al., 2010). HUC-MSCs modified to express BDNF were also found to improve neurological function and increase NSE-positive cells while decrease GFAP-positive cells and number of apoptosis cells after being delivered into the edge of lesion in athymic mice brain injury model (Zhang et al., 2009 as cited in Fan et al., 2011). In addition hUC-MSCs can be genetically modified to express biologically active human factor IX and serve as an efficient drug delivery vehicle for somatic gene therapy of hemophilia B (Chen et al., 2009 as cited in Fan et al., 2011). hUC-MSCs might have a migratory capacity toward glioma cells and therefore can also serve as a

vector for gene therapy against brain tumors. Soluble factors including chemokines or growth factors expressed and released by glioma cells mediate the tropism of MSCs for gliomas. In the study by Park et al. (2011) the overexpression of the stromal cell-derived factor-1α (SDF-1α) receptor, chemokine receptor 4 (CXCR4), on hUCB-MSCs resulted in enhanced migratory capacity of MSCs toward gliomas. Furthermore, CXCR4 transfected hUCB-MSCs (hMSCs-CXCR4) showed a stronger migration capacity toward glioma cells in vitro compared with control MSCs, and also exhibited enhanced migration to glioma cells in an intracranial human malignant glioma xenograft model. These results indicate that SDF-1α/CXCR4 could be involved in recruitment of hUCB-MSCs to glioma cells and that overexpression of CXCR4 may be a useful tool for stem cell-based glioma therapy (Park et al., 2011). It has also been suggested that the genetic alteration of stem cells can function as a mechanism which could deliver specific gene products to parts of tissue ischemia to boost the repair process (Byun et al., 2001; Melo et al., 2004; Yi et al., 2006 as cited in Goldberg et al., 2007) and it was shown to be associated with restored physiological functions after AMI (Chen et al., 2005 as cited in Arien-Zakay et al., 2010). In preclinical studies, UCB CD34+ transfected with VEGF or Ang-1 have been shown to enhance the process of tissue repair in a murine LAD ligation model (Goldberg et al., 2007) and UCB MNCs modified to express VEGF have demonstrated their efficiency in a hindlimb model of chronic ischemia (Ikeda et al., 2004 as cited in Goldberg et al., 2007). Additionally, autologous CB CD 34+ transduced with human adenosine deaminase (ADA) gene were infused in children with ADA deficiency (Kohn et al., 1995 as cited in Lu et al., 1996).

8. Cord blood stem cells transplantation: Advantages and disadvantages

The UCB as a source of human stem cells has proved to be highly advantageous as compared to peripheral blood and bone marrow. UCB has more hematopoietic stem cells per volume than PB and BM (Rogers & Casper, 2004) and the immature hematopoietic stem cells and progenitor cells from cord blood seem to be of good quality (Lu et al., 1996). Stem cells remain in the placenta and umbilical cord after delivery and an average of 120 ml of blood can be collected without risking the health of either the mother or the baby (Rogers & Casper, 2004). Cord blood harvesting is thus simple, easy, painless and non-invasive for the donor, contrary to BM aspiration and consequently there are more potential donors than for BM (Malgieri et al., 2010). Moreover, the procedure does not involve ethical and technical issues (Wu et al., 2009 as cited in Malgieri et al., 2010). Different from BM transplants which require general anaesthesia and surgical transfer of the donor marrow to the recipient, UCB stem cell transplantation is a simple intravenous infusion of the hematopoietic stem cells, which find their way to the BM for engraftment (Rogers & Casper, 2004). Moreover, due to the fact that cord blood units are priory stored in public and private UCB banks (Malgieri et al., 2010) they are quickly available, an advantage which is crucial to high-risk patients with unstable disease (Barker et al., 2002 as cited in Barker & Wagner, 2003), whereas BM has to be collected from the donor just before transplantation and there is always the risk of last minute consent refusal (Malgieri et al., 2010). Successful transplantation require allogeneic grafts matching most, if not all, of the six to ten major HLA antigens between host and donor (Malgieri et al., 2010). The majority of the CB transplantations have been performed to treat patients with malignant and non-malignant diseases (Ballen et al., 2001 as cited in Rogers & Casper, 2004) and many of these originated from sibling donors with partial or complete HLA matching. The incidence of acute GVHD has been lower than the expected

rate in a pediatric population who received transplants with BM. Interestingly, acute GVHD has not been related to the degree of HLA matching, thus proving that the use of cord blood itself is responsible for the lowered risk (Gluckman et al., 2001; Yu et al., 2001 as cited in Rogers & Casper, 2004). Consequently, the HLA type does not have to be a perfect match for allogeneic cord blood transplantation because the possibility that these cells induce immunological reactions is lower than in the case of BM cells (Malgieri et al., 2010). For a given HLA match, the risk of GVHD is lower with UCBT than with BMT, thus allowing UCBT with 1-2 antigen HLA-mismatched (Barker & Wagner, 2003). The UCB reduced graft-versus-host reactivity when compared with adult-derived marrow grafts can be explained through the relative immune immaturity of the newborn cells (MSCs and hematopoietic stem cells) (Malgieri et al., 2010). In this way, the donor poll could be extended and patients belonging to ethnic minorities could be better represented on BM donor registries. Moreover, UCB does not imply risk for the donor or donor attrition and has the advantage of lower cytomegalovirus (CMV) transmission.

The major disadvantage of UCB is the limited cell dose, which is of particular relevance to the application of UCB transplants to adults (Rogers & Casper, 2004). Moreover, while processing, testing and freezing the collected CB in a cord blood bank, there is usually a 10-20% loss from the initial blood volume and cell dose (Stanevsky et al., 2009). Moreover, there is an increased risk of contamination with any cell culture manipulation (Malgieri et al., 2010) and it appears that MSCs from UCB have lower success rate of isolation if compared with MSCs from BM (63% vs 100%) (Kern et al., 2006 as cited in Malgieri et al., 2010). The less experience, the present insufficient standardization of each steps between different banks, as well as inadequate storage policy, may lead to an even greater cell loss. Given that some of the CB transplants are performed with cell dose near the engraftment threshold, modest loss of potency of a product may have a major impact on clinical outcome. Indeed, delayed engraftment due to low cell dose represents one of the main disadvantages and the main restriction of cord blood transplants (Stanevski et al., 2009). However, several strategies- such as improved collection techniques and more efficient cell processing and cryopreservation methods are developing in order to overcome these obstacles and obtain successful engraftment of adults as well as children. Other limitations include the potential risk of genetic disease transmission and lack of donor recall for collection of stem cells or donor lymphocytes in the URD setting (Barker & Wagner, 2003).

9. Cord blood banking

Currently, there is a huge interest in establishing and developing cord blood banks. Worldwide, more than 400,000 cord blood grafts are available in more than 50 cord blood banks. The cord blood collections are managed by cord blood bank networks named Netcord (www.netcord.org), international registries for outcome data collection named Eurocord (www.eurocord.org) and CIBMTR www.cibmtr.org. Eurocord is an international registry developed by the European Blood and Marrow Transplant group (EBMT), which includes more than 190 transplant centers in 36 countries all performing cord blood transplants. Eurocord is collaborating with EBMT and Netcord banks to gather clinical information and monitor all patients transplanted in or outside Europe with Netcord units. The Netcord group was established in 1998 to provide good practice in umbilical cord blood storage, facilitate donor search, improve the quality of the grafts, standardize excellence criteria on an international scale and importantly, to establish procedures for bank

accreditation in collaboration with FACT (Foundation on Accreditation in Cell Therapy). All the practical aspects of cord blood banking, such as mother informed consent, collection techniques, labelling and identification, infectious disease and genetic disease testing, HLA typing, methodology of cell processing, cryopreservation, transportation and release have been extensively published. All these aspects are detailed in the last version of the Netcord-FACT Standards (www.factwebsite.org).

Currently, there are increasing numbers of international exchanges of cord blood units. Profit entities such as private banks, store directed donations collected by obstetricians from babies born into families who intend to use the cord blood for the baby from whom it came (autologous donation) or for another family member in need of future transplantation therapy.

10. Future perspectives

The differentiation potential of UCMSC makes them the perfect candidate for a wide range of clinical applications. UC-derived MSCs showed to be multipotent and their differentiation abilities does not seem to be restricted to the mesodermal lineages, since the cells could be successfully induced to neurones, pancreatic and liver cells. Latest evidences suggest, that UC-MSC populations contain a subset of primitive cells. Therefore, the identification and characterization of these sub-populations in the future is of decisive importance. Another issue of great clinical importance is the question of whether the differentiation potential of the isolated populations is dependent on their location in the UC-tissue. Recently, MSCs markers were developed and may be helpful in this matter.

Additionally, first in vitro and in vivo animal studies showed immune-privileged and immune-modulatory properties of UC-derived MSCs. Due to immune-modulatory properties low levels of rejection were observed in all reports of in vitro transplantation experiments and good results in tissue engraftments were noted. Therefore further studies and clinical trials will clarify whether the in vivo properties of UC-derived MSCs after transplantation exhibits same effects.

One of the ambitious aims of regenerative medicine is the engineering of tissue in vitro. Few but very promising applications of UC-derived MSCs have been reported in this field. Biopolymer scaffolds, mechanical strain approaches, or 3D bioreactors for tissue generation, which were successfully applied with MSCs from other sources will be of great clinical interest in the future.

In the effort to overcome incurable diseases, it may only be a question of time until UC-derived MSCs will be successfully used for clinical and tissue engineering applications.

11. References

[1] Anzalone R, Lo Iacono M, Loria T, Di Stefano A, Giannuzzi P, Farina F & La Rocca G.(2010). Wharton's Jelly Mesenchymal Stem Cells as Candidates for Beta Cells Regeneration: Extending the Differentiative and Immunomodulatory Benefits of Adult Mesenchymal Stem Cells for the Treatment of Type 1 Diabetes. *Stem Cell Rev*.n.d.

[2] Arien-Zakay H, Lazarovici P & Nagler A. (2010). Tissue regeneration potential in human umbilical cord blood. Best Practice & Research Clinical Haematology, Vol. 23, No.2, pp. 291-303.

[3] Arien-Zakay H, Lecht S, Bercu MM, Tabakman R, Kohen R, Galski H, Nagler A & Lazarovici P.(2009). Neuroprotection by cord blood neural progenitors involves antioxidants, neurotrophic and angiogenic factors. *Exp Neurol*. Vol.216, No.1, pp.83-94.

[4] Barker J N., Wagner J E. (2003). Umbilical cord blood transplantation: current practice and future innovations. *Critical Reviews in Oncology/Hematology*, Vol.48, No.1, pp. 35-43.

[5] Bartholomew A, Sturgeon C, Siatskas M, Ferrer K, McIntosh K, Patil S, Hardy W, Devine S, Ucker D, Deans R, Moseley A & Hoffman R (2002) Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. *Exp Hematol*. Vol.30, pp.42-48.

[6] Bielorai B, Hughes MR, Auerbach AD, Nagler A, Loewenthal R, Rechavi G & Toren A.(2004). Successful umbilical cord blood transplantation for Fanconi anemia using preimplantation genetic diagnosis for HLA-matched donor. *Am J Hematol*. Vol.77, No.4, pp. 397-9.

[7] Bizzetto R, Bonfim C, Rocha V, Socié G, Locatelli F, Chan K, Ramirez O, Stein J, Nabhan S, Miranda E, Passweg J, de Souza CA & Gluckman E; Eurocord and SAA-WP from EBMT. (2011). Outcomes after related and unrelated umbilical cord blood transplantation for hereditary bone marrow failure syndromes other than Fanconi anemia. *Haematologica.* Vol.96, No.1, pp. 134-41.

[8] Boelens JJ, Rocha V, Aldenhoven M, Wynn R, O'Meara A, Michel G, Ionescu I, Parikh S, Prasad VK, Szabolcs P, Escolar M, Gluckman E, Cavazzana-Calvo M & Kurtzberg J; EUROCORD, Inborn error Working Party of EBMT and Duke University.(2009). Risk factor analysis of outcomes after unrelated cord blood transplantation in patients with hurler syndrome. *Biol Blood Marrow Transplant*. Vol.15, No.5, pp.618-25.

[9] Boelens JJ, Wynn RF, O'Meara A, Veys P, Bertrand Y, Souillet G, Wraith JE, Fischer A, Cavazzana-Calvo M, Sykora KW, Sedlacek P, Rovelli A, Uiterwaal CS & Wulffraat N.(2007). Outcomes of hematopoietic stem cell transplantation for Hurler's syndrome in Europe: a risk factor analysis for graft failure. *Bone Marrow Transplant*. Vol.40, No.3, pp. 225-33.

[10] Botta R, Gao E, Stassi G, Bonci D, Pelosi E, Zwas D, Patti M, Colonna L, Baiocchi M, Coppola S, Ma X, Condorelli G & Peschle C. (2004). Heart infarct in NOD-SCID mice: therapeutic vasculogenesis by transplantation of human CD34+ cells and low dose CD34+KDR+ cells. *FASEB J*. Vol.18, No.12, pp.1392-4.

[11] Breymann C, Schmidt D & Hoerstrup SP. (2006). Umbilical cord cells as a source of cardiovascular tissue engineering. *Stem Cell Rev*. Vol.2, No.2, pp.87-92.

[12] Campard D, Lysy PA, Najimi M & Sokal EM.(2008). Native umbilical cord matrix stem cells express hepatic markers and differentiate into hepatocyte-like cells. *Gastroenterology*. Vol. 134, No.3, pp.833-48

[13] Canque B, Camus S, Dalloul A, Kahn E, Yagello M, Dezutter-Dambuyant C, Schmitt D, Schmitt C & Gluckman JC.(2000). Characterization of dendritic cell differentiation pathways from cord blood CD34(+)CD7(+)CD45RA(+) hematopoietic progenitor cells. *Blood* .Vol. 96, No. 12, pp. 3748-56.

[14] Chang YS, Oh W, Choi SJ, Sung DK, Kim SY, Choi EY, Kang S, Jin HJ, Yang YS & Park WS. (2009). Human umbilical cord blood-derived mesenchymal stem cells

attenuate hyperoxia-induced lung injury in neonatal rats. *Cell Transplant*. Vol.18, No.8, pp. 869-86.

[15] Chen MY, Lie PC, Li ZL & Wei X. (2009). Endothelial differentiation of Wharton's jelly-derived mesenchymal stem cells in comparison with bone marrow-derived mesenchymal stem cells. *Exp Hematol*. Vol. 37, No.5, pp.629-40.

[16] Chen N, Hudson JE, Walczak P, Misiuta I, Garbuzova-Davis S, Jiang L, Sanchez-Ramos J, Sanberg PR, Zigova T & Willing AE.(2005). Human umbilical cord blood progenitors: the potential of these hematopoietic cells to become neural. *Stem Cells*. Vol. 23, No.10, pp. 1560-70.

[17] Chung DJ, Choi CB, Lee SH, Kang EH, Lee JH, Hwang SH, Han H, Lee JH, Choe BY, Lee SY & Kim HY. (2009). Intraarterially delivered human umbilical cord blood-derived mesenchymal stem cells in canine cerebral ischemia. *J Neurosci Res*.Vol.87, No.16, pp.3554-67.

[18] Conconi MT, Burra P, Di Liddo R, Calore C, Turetta M, Bellini S, Bo P, Nussdorfer GG & Parnigotto PP.(2006). CD105(+) cells from Wharton's jelly show in vitro and in vivo myogenic differentiative potential. *Int J Mol Med*. Vol.18, No.6, pp.1089-96.

[19] Covas DT, Siufi JL, Silva AR & Orellana MD. (2003). Isolation and culture of umbilical vein mesenchymal stem cells. *Braz J Med Biol Res*, Vol. 36, p.1179-1183.

[20] Devine SM, Cobbs C, Jennings M, Bartholomew A & Hoffman R (2003) Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates. *Blood* Vol.101, pp.2999-3001.

[21] Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, Grisanti S & Gianni AM. (2002). Human bone marrow stromal cells suppress T-lymphocyte proliferationinduced by cellular or nonspecific mitogenic stimuli. *Blood* Vol.99, pp.3838-3843.

[22] Fan CG, Zhang Q-j & Zhou J-r. (2011).Therapeutic Potentials of Mesenchymal Stem Cells Derived from Human Umbilical Cord. *Stem Cell Rev and Rep*, Vol 7, No 1, pp. 195-207

[23] Flynn A, Barry F, & O'Brien T. (2007). UC blood-derived mesenchymal stromal cells: an overview. *Cytotherapy*. Vol. 9, No. 8, pp.717-26.

[24] Fu YS, Cheng YC, Lin MY, Cheng H, Chu PM, Chou SC, Shih YH, Ko MH & Sung MS.(2006). Conversion of human umbilical cord mesenchymal stem cells in Wharton's jelly to dopaminergic neurons in vitro: potential therapeutic application for Parkinsonism. *Stem Cells*. Vol. 24, No.1, pp.115-24.

[25] Fu YS, Shih YT, Cheng YC & Min MY.(2004). Transformation of human umbilical mesenchymal cells into neurons in vitro. *J Biomed Sci.* Vol.11, No.5, pp.652-60.

[26] Gang EJ, Jeong JA, Hong SH, Hwang SH, Kim SW, Yang IH, Ahn C, Han H & Kim H.(2004). Skeletal myogenic differentiation of mesenchymal stem cells isolated from human umbilical cord blood. *Stem Cells*, Vol. 22, No.4, pp.617-624

[27] Goldberg JL, Laughlin MJ & Pompili VJ. (2007). Umbilical cord blood stem cells: implications for cardiovascular regenerative medicine. *J Mol Cell Cardiol*, Vol. 42, No. 5, pp. 912-920.

[28] Haller MJ, Viener HL, Wasserfall C, Brusko T, Atkinson MA & Schatz DA.(2008). Autologous umbilical cord blood infusion for type 1 diabetes. *Exp Hematol*. Vol. 36, No.6, pp.710-5.

[29] Harris D.T. & Rogers I. (2007).Umbilical cord blood: a unique source of pluripotent stem cells for regenerative medicine. *Curr Stem Cell Res Ther,* Vol. 2, pp. 301–309.

[30] Henning RJ, Abu-Ali H, Balis JU, Morgan MB, Willing AE & Sanberg PR.(2004). Human umbilical cord blood mononuclear cells for the treatment of acute myocardial infarction. *Cell Transplant*. Vol.13, No.7-8, pp.729-39.

[31] Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA & Finkel T.(2003). Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J *Med.* Vol.348, No.7, pp.593-600.

[32] Hirata Y, Sata M, Motomura N, Takanashi M, Suematsu Y, Ono M & Takamoto S.(2005). Human umbilical cord blood cells improve cardiac function after myocardial infarction. *Biochem Biophys Res Commun*. Vol.327, No.2, pp.609-14.

[33] Hu SL, Luo HS, Li JT, Xia YZ, Li L, Zhang LJ, Meng H, Cui GY, Chen Z, Wu N, Lin JK, Zhu G & Feng H. (2010). Functional recovery in acute traumatic spinal cord injury after transplantation of human umbilical cord mesenchymal stem cells. *Crit Care Med*. Vol.38, No. 11, pp.2181-9.

[34] Huang GP, Pan ZJ, Jia BB, Zheng Q, Xie CG, Gu JH, McNiece IK & Wang JF. (2007). Ex vivo expansion and transplantation of hematopoietic stem/progenitor cells supported by mesenchymal stem cells from human umbilical cord blood. *Cell Transplant*. Vol.16, No.6, pp.579-85.

[35] Hyslop, L. A., Armstrong, L., Stojkovic, M., & Lako, M. (2005). Human embryonic stem cells: biology and clinical implications. *Expert Reviews in Molecular Medicine, Vol.*7, No.19, pp. 1–21.

[36] Hyun JK & Kim HW.(2010). Clinical and experimental advances in regeneration of spinal cord injury. *J Tissue Eng*. 650857.

[37] Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA & Verfaillie CM. (2002). Pluripotency of mesenchymal stem cells deived from adult marrow. *Nature*, Vol.418, pp. 41-49.

[38] Jung KH, Shin HP, Lee S, Lim YJ, Hwang SH, Han H, Park HK, Chung JH & Yim SV. (2009). Effect of human umbilical cord blood-derived mesenchymal stem cells in a cirrhotic rat model. *Liver Int.* Vol. 29, No.6, pp.898-909

[39] Kadivar M, Khatami S, Mortazavi Y, Shokrgozar MA, Taghikhani M Soleimani M.(2006). In vitro cardiomyogenic potential of human umbilical vein-derived mesenchymal stem cells. *Biochem Biophys Res Commun.* Vol. 340, No. 2, pp.639-47.

[40] Kadner A, Hoerstrup SP, Tracy J, Breymann C, Maurus CF, Melnitchouk S, Kadner G, Zund G & Turina M. (2002). Human umbilical cord cells: a new cell source for cardiovascular tissue engineering. *Ann Thorac Surg.* Vol.74, No. 4, pp.S1422-8.

[41] Kim BO, Tian H, Prasongsukarn K, Wu J, Angoulvant D, Wnendt S, Muhs A, Spitkovsky D & Li RK.(2005). Cell transplantation improves ventricular function after a myocardial infarction: a preclinical study of human unrestricted somatic stem cells in a porcine model. *Circulation*. Vol. 112, No.9 Suppl, pp.I96-104.

[42] Kim JY, Jeon HB, Yang YS, Oh W & Chang JW. (2010). Application of human umbilical cord blood-derived mesenchymal stem cells in disease models. *World J Stem Cells*, Vol.2, No.2, pp.34-38.

[43] Kim SW, Han H, Chae GT, Lee SH, Bo S, Yoon JH, Lee YS, Lee KS, Park HK & Kang KS. (2006). Successful stem cell therapy using umbilical cord blood-derived multipotent

stem cells for Buerger's disease and ischemic limb disease animal model. *Stem Cells*. Vol.24, No.6, pp.1620-6.

[44] Kögler G, Sensken S & Wernet P. (2006). Comparative generation and characterization of pluripotent unrestricted somatic stem cells with mesenchymal stem cells from human cord blood. *Exp Hematol*. Vol.34, No.11, pp.1589-95.

[45] Kögler G, Sensken S, Airey JA, Trapp T, Müschen M, Feldhahn N, Liedtke S, Sorg RV, Fischer J, Rosenbaum C, Greschat S, Knipper A, Bender J, Degistirici O, Gao J, Caplan AI, Colletti EJ, Almeida-Porada G, Müller HW, Zanjani E & Wernet P.(2004). A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential.*J Exp Med*. Vol. 200, No.2, pp.123-35.

[46] Krause DS, Theise ND, Cottler MI et al. (2001). Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell*, Vol.105, No. 3, pp. 369-377.

[47] Kuh SU, Cho YE, Yoon DH, Kim KN & Ha Y. (2005). Functional recovery after human umbilical cord blood cells transplantation with brain-derived neutrophic factor into the spinal cord injured rat. *Acta Neurochir (Wien)*. Vol.147, No.9, pp. 985-92.

[48] La Rocca G, Anzalone R, Corrao S, Magno F, Loria T, Lo Iacono M, Di Stefano A, Giannuzzi P, Marasa L, Cappello F, Zummo G & Farina F. (2009). Isolation and characterization of Oct-4+/HLA-G+ mesenchymal stem cells from human umbilical cord matrix: differentiation potential and detection of new markers. *Histochem Cell Biol*, Vol. 131, pp. 267-282.

[49] Laughlin MJ, Barker J, Bambach B, Koc ON, Rizzieri DA, Wagner JE, Gerson SL, Lazarus HM, Cairo M, Stevens CE, Rubinstein P & Kurtzberg J.(2001). Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. *N Engl J Med*, Vol. 344, No.24, pp.1815-22.

[50] Le Blanc K, Rasmusson I, Sundberg B, Gotherstrom C, Hassan M, Uzunel M & Ringden O (2004) Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet*, Vol.363, pp.1439-1441.

[51] Lee HJ, Lee JK, Lee H, Carter JE, Chang JW, Oh W, Yang YS, Suh JG, Lee BH, Jin HK & Bae JS. (2010). Human umbilical cord blood-derived mesenchymal stem cells improve neuropathology and cognitive impairment in an Alzheimer's disease mouse model through modulation of neuroinflammation. *Neurobiol Aging*.n.d.

[52] Lee HJ, Lee JK, Lee H, Shin JW, Carter JE, Sakamoto T, Jin HK & Bae JS. (2010). The therapeutic potential of human umbilical cord blood-derived mesenchymal stem cells in Alzheimer's disease. *Neurosci Lett.* Vol.481, No.1, pp. 30-5.

[53] Leor J, Guetta E, Feinberg MS, Galski H, Bar I, Holbova R, Miller L, Zarin P, Castel D, Barbash IM & Nagler A.(2006). Human umbilical cord blood-derived CD133+ cells enhance function and repair of the infarcted myocardium. *Stem Cells*. Vol. 24, No.3, pp.772-80.

[54] Li M, Zhang SZ, Guo YW, Cai YQ, Yan ZJ, Zou Z, Jiang XD, Ke YQ, He XY, Jin ZL, Lu GH & Su DQ. (2010). Human umbilical vein-derived dopaminergic-like cell transplantation with nerve growth factor ameliorates motor dysfunction in a rat model of Parkinson's disease. *Neurochem Res*. Vol.35, No.10, pp.1522-9.

[55] Limbert C, Päth G, Jakob F & Seufert J.(2008). Beta-cell replacement and regeneration: Strategies of cell-based therapy for type 1 diabetes mellitus. *Diabetes Res Clin Pract*.Vol.79, No.3, pp. 389-99.

[56] Lin SZ, Chang YJ, Liu JW, Chang LF, Sun LY, Li YS, Luo GH, Liao CH, Chen PH, Chen TM, Lee RP, Yang KL, Harn HJ & Chiou TW. (2010). Transplantation of human Wharton's Jelly-derived stem cells alleviates chemically induced liver fibrosis in rats. *Cell Transplant*. Vol.19, No.11, pp. 1451-63.

[57] Liu Y, Mu R, Wang S, Long L, Liu X, Li R, Sun J, Guo J, Zhang X, Guo J, Yu P, Li C, Liu X, Huang Z, Wang D, Li H, Gu Z, Liu B & Li Z.(2010). Therapeutic potential of human umbilical cord mesenchymal stem cells in the treatment of rheumatoid arthritis. *Arthritis Res Ther*.Vol.12, No.6, pp. R210.

[58] Lu L, Shen RN & Broxmeyer HE. (1996). Stem cells from bone marrow, umbilical cord blood and peripheral blood for clinical application: current status and future application. *Crit Rev Oncol Hematol.* Vol.22, No.2, pp. 61-78.

[59] Lu LL, Liu YJ, Yang SG, Zhao QJ, Wang X, Gong W, Han ZB, Xu ZS, Lu YX, Liu D, Chen ZZ & Han ZC. (2006). Isolation and characterization of human umbilical cord mesenchymal stem cells with hematopoiesis-supportive function and other potentials. *Haematologica*. Vol. 91, No.8, pp. 1017-26.

[60] Ma N, Ladilov Y, Moebius JM, Ong L, Piechaczek C, Dávid A, Kaminski A, Choi YH, Li W, Egger D, Stamm C & Steinhoff G.(2006). Intramyocardial delivery of human CD133+ cells in a SCID mouse cryoinjury model: Bone marrow vs. cord blood-derived cells. *Cardiovasc Res*. Vol.71, No.1, pp.158-69.

[61] Ma N, Stamm C, Kaminski A, Li W, Kleine HD, Müller-Hilke B, Zhang L, Ladilov Y, Egger D & Steinhoff G. (2005). Human cord blood cells induce angiogenesis following myocardial infarction in NOD/scid-mice. *Cardiovasc Res*. Vol.66, No.1, pp.45-54.

[62] Malgieri A, Kantzari E, Patrizi MP, Gambardella S. (2010). Bone marrow and umbilical cord blood human mesenchymal stem cells: state of the art. *Int J Clin Exp Med*, Vol. 3, No. 4, pp.248-269.

[63] Mara CS, Duarte AS, Sartori A, Luzo AC, Saad ST & Coimbra IB. (2010). Regulation of chondrogenesis by transforming growth factor-beta 3 and insulin-like growth factor-1 from human mesenchymal umbilical cord blood cells. *J Rheumatol*. Vol. 37, No.7, pp. 1519-26.

[64] Mareschi K, Ferrero I, Rustichelli D, Aschero S, Gammaitoni L, Aglietta M, Madon E & Fagioli F. (2006). Expansion of mesenchymal stem cells isolated from pediatric and adult donor bone marrow. *J Cell Biochem*. Vol.97, No.4, pp.744-54.

[65] Martin-Rendon E, Sweeney D, Lu F, Girdlestone J, Navarrete C & Watt SM.(2008). 5-Azacytidine-treated human mesenchymal stem/progenitor cells derived from umbilical cord, cord blood and bone marrow do not generate cardiomyocytes in vitro at high frequencies. *Vox Sang*. Vol. 95, No.2, pp.137-48.

[66] Matsuzuka T, Rachakatla RS, Doi C, Maurya DK, Ohta N, Kawabata A, Pyle MM, Pickel L, Reischman J, Marini F, Troyer D & Tamura M.(2010). Human umbilical cord matrix-derived stem cells expressing interferon-beta gene significantly attenuate bronchioloalveolar carcinoma xenografts in SCID mice. *Lung Cancer*.Vol.70, No.1, pp. 28-36.

[67] Mitchell KE, Weiss ML, Mitchell BM, Martin P, Davis D, Morales L, Helwig B, Beerenstrauch M, Abou-Easa K, Hildreth T, Troyer D & Medicetty S.(2003). Matrix cells from Wharton's jelly form neurons and glia. *Stem Cells*, Vol. 21, No. 1, pp.50-60.

[68] Moodley Y, Atienza D, Manuelpillai U, Samuel CS, Tchongue J, Ilancheran S, Boyd R & Trounson A.(2009). Human umbilical cord mesenchymal stem cells reduce fibrosis of bleomycin-induced lung injury. *Am J Pathol*.Vol.175, No.1, pp. 303-13.

[69] Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, Pasumarthi KB, Virag JI, Bartelmez SH, Poppa V, Bradford G, Dowell JD, Williams DA & Field LJ.(2004). Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature*. Vol.428, No.6983, pp.664-8.

[70] Park SA, Ryu CH, Kim SM, Lim JY, Park SI, Jeong CH, Jun JA, Oh JH, Park SH, Oh W & Jeun SS. (2011). CXCR4-transfected human umbilical cord blood-derived mesenchymal stem cells exhibit enhanced migratory capacity toward gliomas. *Int J Oncol*. Vol.38, No.1, pp.97-103.

[71] Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M, Oz MC, Hicklin DJ, Witte L, Moore MA & Rafii S. (2000). Expression of VEGFR-2 and AC133 by circulating human CD34 (+) cells identifies a population of functional endothelial precursors. *Blood*. Vol. 95, No. 3, pp. 952-8

[72] Pesce M, Orlandi A, Iachininoto MG, Straino S, Torella AR, Rizzuti V, Pompilio G, Bonanno G, Scambia G & Capogrossi MC.(2003). Myoendothelial differentiation of human umbilical cord blood-derived stem cells in ischemic limb tissues. *Circ Res*. Vol. 93, No.5 pp.e51-62.

[73] Phuc PV, Nhung TH, Loan DT, Chung DC & Ngoc PK.(2011). Differentiating of banked human umbilical cord blood-derived mesenchymal stem cells into insulin-secreting cells. *In Vitro Cell Dev Biol Anim*. Vol.47, No.1, pp.54-63.

[74] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S & Marshak DR. (1999). Multilineage potential of mesenchymal cells. *Science*, Vol. 284, No. 5411, pp.143-147.

[75] Prasad VK & Kurtzberg J. (2010). Transplant outcomes in mucopolysaccharidoses. *Semin Hemato*l. Vol.47, No.1, pp.59-69.

[76] Prasad VK & Kurtzberg J.(2010). Cord blood and bone marrow transplantation in inherited metabolic diseases: scientific basis, current status and future directions. *Br J Haematol*. Vol. 148, No. 3, pp.356-72.

[77] Reddi AS, Kuppasani K & Ende N. (2010). Human umbilical cord blood as an emerging stem cell therapy for diabetes mellitus. *Curr Stem Cell Res The*r. Vol. 5, No.4, pp.356-61.

[78] Ringden O, Uzunel M, Rasmusson I, Remberger M, Sundberg B, Lonnies H, Marschall HU, Dlugosz A, Szakos A, Hassan Z, Omazic B, Aschan J, Barkholt L & Le Blanc K. (2006). Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. *Transplantation*, Vol.81, pp.1390-1397.

[79] Rizvanov AA, Guseva DS, Salafutdinov II, Kudryashova NV, Bashirov FV, Kiyasov AP, Yalvaç ME, Gazizov IM, Kaligin MS, Sahin F, Mukhamedyarov MA, Palotás A & Islamov RR. (2011). Genetically modified human umbilical cord blood cells expressing vascular endothelial growth factor and fibroblast growth factor 2 differentiate into glial cells after transplantation into amyotrophic lateral sclerosis transgenic mice. *Exp Biol Med (Maywood)*. Vol.236, No.1, pp.91-8.

[80] Rogers I & Casper R F. (2004). Umbilical cord blood stem cells. *Best Practice & Research Clinical Obstetrics and Gynaecology*, Vol. 18, No. 6, pp. 893-908.

[81] Rogers I, Yamanaka N, Bielecki R, Wong CJ, Chua S, Yuen S & Casper RF. (2007). Identification and analysis of in vitro cultured CD45-positive cells capable of multi-lineage differentiation. *Exp Cell Res*. Vol. 313, No. 9, pp.1839-52.

[82] Romanov YA, Svintsitskaya VA & Smirnov VN. (2003). Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord.*Stem Cells*, Vol. 21, No. 1, pp. 105-110.

[83] Roobrouck VD, Ulloa-Montoya F & Verfaillie CM. (2008). Self-renewal and differentiation capacity of young and aged stem cells. *Experimental Cell Research*, Vol. 314, No.9, pp. 1937-1944.

[84] Roobrouck VD, Ulloa-Montoya F & Verfaillie CM.(2008). Self-renewal and differentiation capacity of young and aged stem cells. *Exp Cell Res.* Vol.314, No.9, pp.1937-44.

[85] Roura S, Farré J, Hove-Madsen L, Prat-Vidal C, Soler-Botija C, Gálvez-Montón C, Vilalta M & Bayes-Genis A. (2010). Exposure to cardiomyogenic stimuli fails to transdifferentiate human umbilical cord blood-derived mesenchymal stem cells. *Basic Res Cardiol*. Vol. 105, No. 3, pp. 419-30.

[86] Sanberg PR, Willing AE, Garbuzova-Davis S, Saporta S, Liu G, Sanberg CD, Bickford PC, Klasko SK & El-Badri NS. (2005). Umbilical cord blood-derived stem cells and brain repair. *Ann N Y Acad Sci.* Vol.1049, pp.67-83.

[87] Schneider RK, Puellen A, Kramann R, Raupach K, Bornemann J, Knuechel R, Pérez-Bouza A & Neuss S. (2010). The osteogenic differentiation of adult bone marrow and perinatal umbilical mesenchymal stem cells and matrix remodelling in three-dimensional collagen scaffolds. *Biomaterials*. Vol. 31, No.3, pp. 467-80.

[88] Sellamuthu S, Manikandan R, Thiagarajan R, Babu G, Dinesh D, Prabhu D & Arulvasu C.(2011). In vitro trans-differentiation of human umbilical cord derived hematopoietic stem cells into hepatocyte like cells using combination of growth factors for cell based therapy. *Cytotechnology*. n.d.

[89] Shaw PH, Haut PR, Olszewski M & Kletzel M.(1999). Hematopoietic stem-cell transplantation using unrelated cord-blood versus matched sibling marrow in pediatric bone marrow failure syndrome:one center's experience. *Pediatric Transplantation*, Vol. 3, No. 4, pp. 315-321.

[90] Silani V, Cova L, Corbo M, Ciammola A & Polli E. (2004). Stem-cell therapy for amyotrophic lateral sclerosis. *Lancet*. Vol.364, No.9429, pp.200-2.

[91] Smith AR & Wagner JE. (2009). Alternative Hematopoietic Stem Cell Sources for Transplantation: Place of Umbilical Cord Blood. Br J Haematol. Vol.147, No.2, pp. 246–261.

[92] Sobani ZA, Quadri SA & Enam SA.(2010). Stem cells for spinal cord regeneration: Current status. *Surg Neurol Int.* Vol.25, No.1, pp.93.

[93] Sodian R, Lueders C, Kraemer L, Kuebler W, Shakibaei M, Reichart B, Daebritz S & Hetzer R. (2006). Tissue engineering of autologous human heart valves using cryopreserved vascular umbilical cord cells. *Ann Thorac Surg*. Vol.81, No.6, 2207-16.

[94] Stanevski A, Goldstein G & Nagler A. (2009). Umbilical cord blood transplantation: Pros, cons and beyond. *Blood Reviews*, Vol. 23, pp. 199-204.

[95] Wang HS, Shyu JF, Shen WS, Hsu HC, Chi TC, Chen CP, Huang SW, Shyr YM, Tang KT & Chen TH.(2010). Transplantation of insulin producing cells derived from umbilical cord stromal mesenchymal stem cells to treat NOD mice. *Cell Transplant*.n.d.

[96] Wang L, Tran I, Seshareddy K, Weiss ML & Detamore MS. (2009). A comparison of human bone marrow-derived mesenchymal stem cells and human umbilical cord-derived mesenchymal stromal cells for cartilage tissue engineering. *Tissue Eng Part A*. Vol.15, No. 8, pp. 2259-66.

[97] Weiss ML, Medicetty S, Bledsoe AR, Rachakatla RS, Choi M, Merchav S, Luo Y, Rao MS, Velagaleti G & Troyer D. (2006). Human umbilical cord matrix stem cells: preliminary characterization and effect of transplantation in a rodent model of Parkinson's disease. *Stem Cells,* Vol. 24, pp. 781-792.

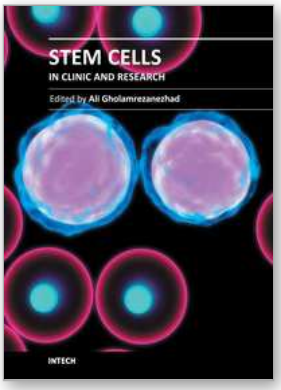
[98] Wobus, Anna M.; Boheler, Kenneth. Stem Cells (Eds.) 1st ed. 2006. 2nd printing, 2006, XII, 414 p. 53 illus., 18 in color., Softcover ISBN: 978-3-540-77854-7

[99] Wu KH, Zhou B, Lu SH, Feng B, Yang SG, Du WT, Gu DS, Han ZC & Liu YL. (2007). In vitro and in vivo differentiation of human umbilical cord derived stem cells into endothelial cells. *J Cell Biochem.* Vol. 100, No. 3, pp.608-16.

[100] Yan-Wu G, Yi-Quan K, Ming L, Ying-Qian C, Xiao-Dan J, Shi-Zhong Z, Wang-Ming Z & Chuan-Zhi D. (2011). Human Umbilical Cord-Derived Schwann-Like Cell Transplantation Combined with Neurotrophin-3 Administration in Dyskinesia of Rats with Spinal Cord Injury. *Neurochem Res*. n.d.

[101] Zhang X, Hirai M, Cantero S, Ciubotariu R, Dobrila L, Hirsh A, Igura K, Satoh H, Yokomi I, Nishimura T, Yamaguchi S, Yoshimura K, Rubinstein P & Takahashi TA. (2011). Isolation and characterization of mesenchymal stem cells from human umbilical cord blood: Reevaluation of critical factors for successful isolation and high ability to proliferate and differentiate to chondrocytes as compared to mesenchymal stem cells from bone marrow and adipose tissue. *J Cell Biochem*. Vol.112, No.4, pp. 1206-18.

[102] Zhao Q, Ren H, Li X, Chen Z, Zhang X, Gong W, Liu Y, Pang T & Han ZC.(2009). Differentiation of human umbilical cord mesenchymal stromal cells into low immunogenic hepatocyte-like cells. *Cytotherapy*. Vol.11, No.4, pp.414-26.



Stem Cells in Clinic and Research

Edited by Dr. Ali Gholamrezanezhad

ISBN 978-953-307-797-0
Hard cover, 804 pages
Publisher InTech
Published online 23, August, 2011
Published in print edition August, 2011

Based on our current understanding of cell biology and strong supporting evidence from previous experiences, different types of human stem cell populations are capable of undergoing differentiation or trans-differentiation into functionally and biologically active cells for use in therapeutic purposes. So far, progress regarding the use of both in vitro and in vivo regenerative medicine models already offers hope for the application of different types of stem cells as a powerful new therapeutic option to treat different diseases that were previously considered to be untreatable. Remarkable achievements in cell biology resulting in the isolation and characterization of various stem cells and progenitor cells has increased the expectation for the development of a new approach to the treatment of genetic and developmental human diseases. Due to the fact that currently stem cells and umbilical cord banks are so strictly defined and available, it seems that this mission is investigationally more practical than in the past. On the other hand, studies performed on stem cells, targeting their conversion into functionally mature tissue, are not necessarily seeking to result in the clinical application of the differentiated cells; In fact, still one of the important goals of these studies is to get acquainted with the natural process of development of mature cells from their immature progenitors during the embryonic period onwards, which can produce valuable results as knowledge of the developmental processes during embryogenesis. For example, the cellular and molecular mechanisms leading to mature and adult cells developmental abnormalities are relatively unknown. This lack of understanding stems from the lack of a good model system to study cell development and differentiation. Hence, the knowledge reached through these studies can prove to be a breakthrough in preventing developmental disorders. Meanwhile, many researchers conduct these studies to understand the molecular and cellular basis of cancer development. The fact that cancer is one of the leading causes of death throughout the world, highlights the importance of these researches in the fields of biology and medicine.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821