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REVIEW ARTICLE

Stem and progenitor cells in human umbilical cord blood

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Abstract Both stem cells and progenitor cells are present in umbilical cord blood (UCB) at a high frequency, making these cells a major target population for experimental and clinical studies. As the use of autologous or allogeneic hematopoietic stem cell transplantation in the treatment of various diseases has grown rapidly in recent years, the concept of UCB banking for future use has drawn increasing interest. Stem and progenitor cells derived from UCB offer multiple advantages over adult stem cells, such as their immaturity (which may play a significant role in reducing rejection after transplantation into a mismatched host) and ability to produce large quantities of homogeneous tissue or cells. These cells can also differentiate across tissue lineage boundaries into neural, cardiac, epithelial, hepatic, and dermal tissues. Human UCB provides an alternative cell source that is ethically acceptable and widely supported by the public. This paper summarizes the characteristics of human UCB-derived stem and progenitor cells and their potential therapeutic use for tissue and cell regeneration.

Keywords Umbilical cord blood · Stem cells · Progenitor cells

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1 Introduction

Stem cells (SCs) are capable of self-replication and differentiation into one or several specific cell types. Two types of SCs have been identified: embryonic stem cells (ESCs) in the inner cell mass of the early embryo and adult (or somatic) tissue-specific stem cells. Adult SCs are present in the bone marrow (BM) [1], blood [2], cornea and retina [3], skin [4], skeletal muscles [5], dental pulp [6], liver [7], and brain [8]. As adult SCs can be propagated in large quantities without losing their ability to differentiate into different tissue types, they represent a highly valuable resource for the development of cellular therapies [9].

Umbilical cord blood (UCB), the blood remaining in the umbilical cord and placenta after birth, is usually regarded as medical waste and is routinely discarded. Recently, however, UCB has been widely used as a rich, ethically acceptable, source of SCs with high regeneration and differentiation potentials. UCB is easily available, can be routinely harvested without risk to the donor, and is rarely contaminated with infectious agents such as cytomegalovirus (CMV) [10]. UCB has been used as a source of hematopoietic SCs (HSCs) in various clinical settings since 1988 [11]. Since then, hundreds of thousands of UCB collections have been frozen and stored throughout the world in anticipation of their potential use for the treatment of various disorders. Consequently, investigation into the potential of cryopreserved UCB as a source of stem cells is considered vital for future cell-based therapies.

In addition to HSCs, other potential SCs, such as mesenchymal SCs (MSCs) [12, 13], unrestricted somatic SCs (USSCs) [14], cord blood-derived embryonic-like SCs (CBEs) [15], and UCB-derived multipotent progenitor cells (MPCs) [16] have been isolated from UCB and characterized according to their morphology, immunophenotypes,

and proliferation and differentiation potentials. Because UCB-derived cells are regarded as more primitive than BM-derived cells, they are a more suitable cell source for cell-based therapies, regenerative medicine, and tissue engineering.

2 Hematopoietic stem cells

Since the first UCB transplantation was performed in 1988 in a child with Fanconi's anemia [11], UCB has become a safe and accepted mode of HSC transplantation (HSCT). In addition to its low exposure to viruses, UCB transplantation carries a reduced risk of severe acute graft-versus-host disease (GvHD), which can occur when one or two human leukocyte antigen (HLA)-mismatched unrelated donor transplants are performed. In contrast, BM transplantation requires strict histocompatibility between donors and recipients.

In the cord blood, HSCs are a heterogeneous population of immature hematopoietic precursor cells that occur rarely (at a frequency of approximately 1 in 10^4 to 1 in 10^5 cells postnatally). They are multipotent, and can differentiate into any one of 10–11 functional hematopoietic lineages. Consequently, HSCs are capable of repopulating the whole hematopoietic system within the human lifespan [17, 18]. UCB has a higher primitive HSC content than either BM or mobilized peripheral blood, and has a higher proliferative potential with an extended lifespan and longer telomeres [19, 20]. HSCs contain a small population of $CD34^+$, primitive, and pluripotent SCs that can self-renew and generate committed progenitors of both the myeloid and lymphoid compartments. Although CD34 is not a universal marker for UCB-derived SCs [21], it has been used as a convenient marker for human hematopoietic stem or progenitor cells for autologous and allogeneic transplantation, resulting in the reconstitution of all hematopoietic lineages [22]. Most colony-forming cells are found within the $CD34^+$ cell population [23, 24].

While the number of $CD34^+$ HSCs in cord blood is limited, cord blood-derived HSCs obtained following ex vivo expansion [25, 26] or co-infusion of two or more units may serve as a reliable source for HSCT. Transplanting ex vivo-expanded cells reportedly accelerates regeneration. Ex vivo-expanded cells behave differently from freshly isolated cells. For example, they result in delayed engraftment [27] caused either by a lack of sufficient progenitors or by the decreased expression levels of homing and related proteins, along with reduced amplification.

Proteomic surveys of UCB-derived $CD34^+$ cells are designed to investigate how protein expression varies at different maturation stages within the hematopoietic hierarchy [28]. More than a dozen proteins have been identified that are expressed only on $CD34^+$ cells. Among these, the prostatic binding protein (PBP/RKIP) has a predominant role in proliferation and homing regulatory events [29–31]. This suggests that assessing the cord blood-derived, primitive $CD34^+$ cells is important for early engraftment in HSCT. Although HSCT has been used most often to treat malignant disease [32], UCB has been successfully used to treat nonmalignant diseases such as aplastic anemia and Fanconi's anemia [33]. In addition, many investigators have observed improved engraftment after co-transplantation of MSCs and cord blood-derived HSCs [34–36].

3 Mesenchymal stem cells

The isolation of MSCs is primarily achieved by plastic adherence, followed by growth under specific culture conditions, such as special culture media containing defined growth factors. To date, the most popular source of MSCs has been the BM. However, BM aspiration is an invasive procedure. Moreover, the differentiation potential of BM-MSCs decreases with age. Recently, MSCs have been isolated from various sources, including UCB. Although some investigators failed to isolate MSCs from UCB-derived cells [37], other recent attempts have been successful [12, 13, 38, 39]. MSCs are capable of differentiating into osteoblasts, chondrocytes, adipocytes, and myogenic and neuronal cells.

We, as well as others [38, 39], have provided strong evidence for the presence of circulating non-HSCs, including MSCs, in human UCB. These UCB-MSCs were strongly positive for MSC-specific cell surface markers such as CD105 (SH2), CD73 (SH3, SH4), and CD166 (ALCAM), but negative for CD14 (monocyte antigen), CD31 (endothelial cell antigen), CD34 (HSC antigen), CD45 (leukocyte common antigen), and CD86 (a co-stimulatory molecule). The cell surface antigen profile of UCB-MSCs was essentially the same as that for BM-MSCs [40, 41]. The UCB-MSCs were more proliferative than BM-MSCs during early passages, while the total cell number of expanded UCB-MSCs in long-term cultures was lower than that of BM-MSCs [42]. These phenomena can be explained by both intrinsic and/or extrinsic factors. UCB reportedly contains more primitive SCs than BM, which may explain why UCB-MSCs are more proliferative than BM-MSCs during early growth stages. The difference in the total cell number after long-term culture may be related to other inherent characteristics of the UCB and BM samples, including the frequency of MSCs within the cell populations.

The frequency of MSCs in cryopreserved UCB units has not been clearly defined. Previous studies using colony-forming unit fibroblast (CFU-F) culture as a surrogate

assay reported that the frequency of BM-MSCs in adults was one in 3.4×10^4 cells. However, fresh UCB and peripheral blood SCs (PBSCs) did not produce CFU-F at all [37, 43]. It is possible that MSCs in cryopreserved UCBs are present at a very low frequency relative to MSCs in BM. Since a few UCB-MSCs were more proliferative than BM-MSCs during early passages, the doubling potential of UCB-MSCs may decrease more quickly than that of BM-MSCs after a finite number of doublings. Extrinsic factors affecting growth rate may include differences in the culture conditions required for UCB-MSCs and BM-MSCs cultivation (e.g., medium composition, serum, pH, positive or negative selection, and the efficiency of trypsinization), and the 'human factor' that determines when and how the cultures are passaged. The precise reason for the differences in growth rate between BM-MSCs and UCB-MSCs requires further study.

Clinically, the immunomodulatory properties of MSCs can be used to enhance engraftment and to reduce the incidence of GvHD after allogeneic HSCT [34–36]. We previously demonstrated that UCB-MSCs were capable of suppressing mitogen-induced T cell proliferation to levels similar to those seen with BM-MSCs [44]. The use of UCB-MSCs has a major advantage in that it does not require invasive procedures that could be harmful to the donor. UCB may be a better third-party source of MSCs that can be used universally across the HLA barrier. Several studies have shown that MSCs possess an intrinsic homing ability, migrating to the injured tissues and actively participating in tissue repair. MSCs can repair injured tissue by differentiating into damaged cell types, secreting appropriate cytokines and growth factors, and undergoing cell fusion [45–47]. In addition, MSCs possess the unique ability to suppress immune responses, both in vitro [48, 49] and in vivo [50–52].

MSCs with high proliferative and differentiation potentials are present in UCB. The in vitro isolation, expansion, and characterization of UCB-MSCs will be useful for basic research, and are expected to facilitate the development of therapeutic strategies, such as cellular and genetic therapies.

4 Unrestricted somatic stem cells

Kögler et al. [14] identified a rare population of $CD45^+$/HLA class II-negative SC candidates in UCB, which they termed unrestricted somatic SCs (USSCs). This cell population displayed a robust in vitro proliferative capacity without spontaneous differentiation, but with intrinsic and controllable differentiation into cell types found in mesodermal, endodermal, and ectodermal lineages [14]. In contrast to BM-MSCs [41], USSCs have a wider differentiation potential and different immunophenotypes [53] and mRNA expression profiles.

USSC cultures were initiated from 573 UCB samples with a total generation frequency of 35.4% ($n = 203$) [54]. After 6–20 days, between one and 11 USSC colonies/UCB were detected, which grew into monolayers within 2–3 weeks. No correlation was observed between successful initiation of USSC cultures and the gestational age (>36 weeks), cord blood volume (always >40 mL), number of nucleated cells in the UCB collections ($>2 \times 10^8$), hours elapsed after UCB collection (up to 57 h), or the number of mononuclear cells (MNCs) in the UCB after gradient separation [55]. USSCs can be cultured for >20 passages (equivalent to >40 population doublings) without spontaneous differentiation. USSCs have longer telomeres than BM-MSCs, which may explain their high expansion capacity. They also constitute an easily accessible cell source that has high proliferation capacity without loss of the normal karyotype during cultivation [14, 55].

USSCs are adherent, spindle-shaped cells of 20–25 μm in size. They are negative for CD14, CD33, CD34, CD45, CD49b, CD49c, CD49d, CD49f, CD50, CD62E, CD62L, CD62P, CD106, CD117, glycophorin-A, and HLA-DR. They express high levels of CD13, CD29, CD44, CD49e, CD90, CD105, vimentin, and cytokeratin 8 and 18 and low levels of CD10 and FLK1 (KDR) [14]. USSCs express various transcripts for cytokine receptors, transcription factors, and cell surface markers, including epidermal growth factor receptor, platelet-derived growth factor receptor, insulin-like growth factor receptor, runt-related transcription factor (Runx1), YB1, CD49e, and CD105. The cells are negative for the chondrogenic extracellular protein chondroadherin, the bone-specific markers collagenase X and bone sialoprotein, the liver- and pancreas-specific markers Cyp1A1 and PDX-1, and neural markers such as neurofilament, synaptophysin, tyrosine hydroxylase, and glial fibrillary acid protein [55].

USSCs are capable of differentiating into various lineages in vitro and in vivo, including neuronal cells, osteoblasts, chondrocytes, adipocytes, hematopoietic cells, cardiomyocytes, purkinje fibers, and hepatic cells [14]. Transplantation of USSCs reportedly improves left ventricle (LV) function and prevents scar formation and LV dilation after acute myocardial infarction. Since differentiation, apoptosis, and macrophage mobilization at the infarct site were excluded as underlying mechanisms, paracrine effects most likely account for the observed effect of USSC treatment [56]. In addition, USSCs show increased secretion of vascular endothelial growth factor (VEGF) during osteogenic differentiation, as well as expression of key markers of angiogenesis such as VEGF receptor-2 and platelet/endothelial cell adhesion molecules. When transplanted into a bone defect, USSCs might

support the repair process by pure remineralization and installation of an angiogenic environment [57].

USSCs provide an unlimited source of cellular grafts for therapeutic purposes and exhibit considerable advantages over other cell types. Autologous cells isolated after birth can be stored for individual later use. Further investigations are necessary to analyze the impact of graft-related immune responses and to compare the outcome of xenogeneic and allogeneic USSC transplantation.

5 Cord blood-derived embryonic-like stem cells

McGuckin et al. [15] reported the reproducible production of untransformed adherent human SC populations with an ESC phenotype from UCB, termed cord blood-derived embryonic-like SCs (CBEs) [15, 58]. The CBEs formed embryoid body-like colonies that were immunoreactive for primitive human ESC-specific genes [59], suggesting that CBEs were capable of differentiating into neuronal, hepatic, pancreatic, bone, fat, skeletal muscle, and blood vessel cells [15].

One week after the initial plating of the primary culture, the adherent cell clusters formed embryoid body-like colonies that progressively increased in both size and number. The adherent colonies could be dissociated at week 6 or 7 and reseeded in second-generation liquid cultures. Second-generation CBEs formed embryoid body-like structures with a morphology similar to that of their first-generation progenitor colonies. The CBEs were grown for up to 6 additional weeks and demonstrated an exponential cell proliferation pattern. Second-generation CBE populations significantly expanded (168-fold) from their baseline concentration (10^5 cells/mL) to yield $1.68 \times 10^7 \pm 8.84 \times 10^5$ cells [60].

CBEs are negative for the hematopoietic lineage markers CD45, glycophorin-A, CD38, CD7, CD33, CD56, CD16, CD3, and CD2, and are positive for CD34, CD133, and CD164. The CBE colonies are positive for the embryonic stage-specific antigens SSEA-3 and SSEA-4, but are negative for embryonic antigen-1 (SSEA-1), confirming their undifferentiated phenotype [61]. They also express the ESC transcription factor Oct-4 involved in differentiation inhibition and ESC self-renewal [62]. CBE colonies express the embryonic extracellular matrix components Tra 1-60 and Tra 1-81 [15, 58], which most likely contribute to the colony-like nature of their clustered organization [63].

Multi-lineage progenitor cells (MLPCs^{TM}, an improved cell line commercially available from BioE in Minnesota, USA) are capable of differentiating into bone, fat, skeletal muscle, blood vessels, and liver/pancreatic cells [15]. Neural precursor and cells generated from CBEs may be used for in vitro drug testing and cell-based assays, and potentially for clinical transplantation.

6 Multipotent progenitor cells

While studying whether other SCs are present in either fresh or cryopreserved UCB, our group isolated a novel cell line from an SC population in human UCB [16]. Seeded UCB-derived MNCs formed adherent colonies under optimized culture conditions. Over a 3- to 4-week culture period, the colonies gradually developed into adherent monolayers that exhibited a homogeneous fibroblast-like morphology and immunophenotype, and were highly proliferative. We refer to these cells as UCB-derived multipotent progenitor cells (MPCs) [16].

MPCs were obtained from 95.5% of UCB harvests, without the need for complicated separation procedures. The cells were negative for CD34, CD49a, CD62E, CD73, CD90, CD104, and CD133, and expressed high levels of CD14, CD31, CD44, CD45, and CD54, with variable expression of CD105 and CD166. The surface antigen profile of MPCs was sustained for more than 12 weeks, and MPCs were highly proliferative with a 28-fold increase in cell number at 12 weeks. While the MPCs were negative for CD34 and CD133 at late culture times, these HSC-specific surface markers were fully expressed in the adherent, colonized cells at early culture times. The morphology of the colonized cells closely resembled that of cells in HSC colonies [64]. Thus, isolated MPCs can be derived from HSCs or their precursors in UCB. However, the relationship between MPCs and HSCs in UCB requires further investigation. The MPCs were negative or only weakly positive for MSC-related markers such as CD73 (SH3, SH4), CD105 (SH2), and CD166 (ALCAM). They had a rod-like shape and were relatively small and plump compared with MSCs. Immunophenotypic and morphological data indicated that the MPCs were distinct from stromal MSCs. In addition, CD45 was expressed in MPCs, but not in human USSCs or CBEs. Thus, MPCs are characteristically different from HSCs, MSCs, USSCs, and CBEs (Table 1).

Isolated MPCs were capable of differentiating into three germinal tissue-specific cell types, osteoblasts, myoblasts, endothelial cells, hepatocytes [65], and neuronal cells [16]. In a preclinical study, MPCs promoted functional recovery in rats with spinal cord injury [66]. In addition, transplanted MPCs successfully incorporated into the liver in rat models with hepatic injury, and differentiated into functional hepatocytes that expressed CK-18 and albumin, a hepatocyte-specific marker [67]. Thus, MPCs could potentially serve as a universal allogeneic stem/progenitor cell source for use in the development of SC-based therapies.

Table 1 Immunophenotypic comparison of stem and progenitor cells derived from umbilical cord blood

Cell surface marker	HSC	MSC	USSC	CBE	MPC
CD34	+	−	−	+	−
CD133	+	−		+	−
CD14	−	−	−		+
CD45	+	−	−	−	+
CD44	+	+	+		+
CD54		+	+		+
CD73	−	+			−
CD90	+	+	+		−
CD105	−	+	+		+
CD166		+			+

Values of immunophenotyping were determined by flow cytometry on a FACScan (BD Sciences)

HSC hematopoietic stem cell, *MSC* mesenchymal stem cell, *USSC* unrestricted somatic stem cell, *CBE* cord blood-derived embryonic-like stem cell, *MPC* multipotent progenitor cell, *CD34* and *CD133* hematopoietic stem cell, *CD14* monocyte, *CD45* leukocyte common, *CD44* hyaluronan receptor, *CD73 (SH3, SH4)* mesenchymal stem cell, *CD90 (Thy-1)* Thy-1 membrane glycoprotein precursor, *CD105 (SH2)* mesenchymal stem cell, *CD166* ALCAM (activated leukocyte cell adhesion molecule), + strong positive, − strong negative

7 Conclusions

The use of SCs for cell replacement therapy constitutes a promising approach for the treatment of various diseases and injuries. Ideally, such cells should exhibit two key properties: (1) a high level of proliferation in vitro, allowing production of a large number of cells from a limited amount of donor material, and (2) phenotypic plasticity, facilitating differentiation into various tissue-specific cells. To date, several approaches to SC therapies have been used to treat various diseases. However, many studies using SCs present serious ethical problems related to the destruction of the human embryo and possible teratoma formation in the recipient. Thus, SCs from non-embryonic sources offer a real prospect for clinical intervention in the short term and have been successful for the treatment of over 70 diseases and disorders.

Initially, UCB and BM SCs were used as an intervention in only blood- or immune system-related problems. However, their use in the treatment of genetic diseases and their application to regenerative medicine has opened new possibilities that were previously reserved for only ESCs. Progress in SC therapy has been impeded by the need to develop clinical-grade protocols for both the harvesting and processing of the SC source into a transplantation-suitable form. Determination of the stage at which a SC source should be for transplantation, whether as a primary SC or expanded and/or developed progenitor population and/or fully developed mature cell, is a limiting factor that still requires meticulous research.

In conclusion, various stem and progenitor cells with different characteristics that can be derived from fresh or cryopreserved UCB could be good candidates for use in SC therapy. Other SCs may exist within UCB that are even more primitive and have a higher potential for plasticity. Further studies are warranted to identify the yet unrecognized value of UCB-derived SCs.

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