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Regenerative Therapy and Immune Modulation Using Umbilical Cord Blood–Derived Cells

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A B S T R A C T

Since the first cord blood transplantation in 1988, umbilical cord blood has become an important option as a source of cells for hematopoietic transplantation. Beyond its role in regenerating the blood and immune systems to treat blood diseases and inherited metabolic disorders, the role of nonhematopoietic progenitor cells in cord blood has led to new and emerging uses of umbilical cord blood in regenerative therapy and immune modulation. In this review, we provide an update on the clinical and preclinical studies using cord blood–derived cells such as mesenchymal stromal cells, endothelial-like progenitor cells, and others. We also provide insight on the use of cord blood cells as vehicles for the delivery of therapeutic agents through gene therapy and microvesicle-associated strategies. Moreover, cord blood can provide essential reagents for regenerative applications. Clinical activity using cord blood cells is increasing rapidly and this review aims to provide an important update on the tremendous potential within this fast-moving field.

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INTRODUCTION

Widespread use of human umbilical cord blood (UCB) as a source of hematopoietic stem and progenitor cells for transplantation in patients undergoing treatment for leukemia, other blood disorders, and inherited metabolic diseases has been complemented more recently by the promise of regenerative therapy using nonhematopoietic cells present in cord blood [1]. Major nonhematopoietic cell types that can be readily expanded ex vivo using established methods include mesenchymal stromal cells (MSCs) and endothelial-like vascular progenitors (EPCs) that will be reviewed in greater detail. Moreover, technical hurdles to overcome before more widespread clinical use is realized are discussed and indirect uses of UCB-derived cells are presented to illustrate the broad scope of potential applications in regenerative therapy using umbilical cord blood.

MSCs

MSCs comprise a rare population of multipotent perivascular progenitors capable of supporting hematopoiesis in bone marrow (BM) niches, differentiating into multiple mesenchymal lineages, such as osteogenic, adipogenic, and chondrogenic cells, and possess immune modulatory properties that may be applicable in the treatment of inflammatory or autoimmune conditions [2]. MSCs can be derived from various adult organs including adipose tissue [3], BM [2] or UCB, and placental tissues [4,5]. Indeed, MSCs have been used increasingly in clinical trials with the recent development of consensus definitions [6] and the use of standard functional assays that meet regulatory requirements. Moreover, national blood operators in some jurisdictions have embraced manufacturing of MSCs for human use [7,8] and an increasing number of academic institutions and private companies are investing in cell manufacturing facilities to accommodate MSC-based clinical applications.

Although most studies use marrow-derived MSCs, the derivation of MSCs from UCB was first identified in the early 2000s [4,9], but they could only be expanded from a minority of samples [10]. Sharing morphological and phenotypic similarities with BM-derived MSCs, UCB-derived MSCs display a predominantly plastic-adherent fibroblast-like morphology in vitro with an immunophenotype that includes surface expression of CD73, CD90, and CD105 as well as more recently recognized markers CD44, CD146, and CD166 that may allow further enrichment of cells within

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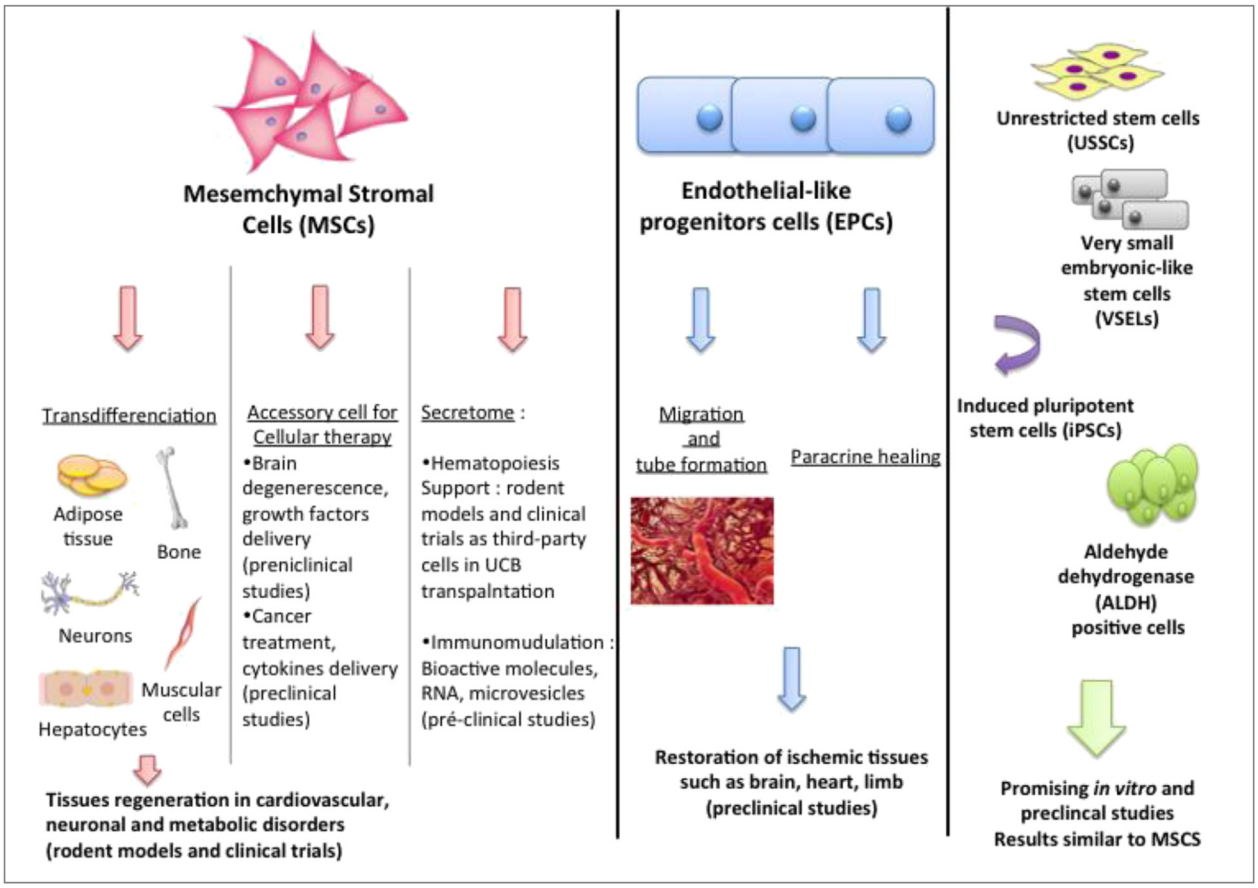

Figure 1. UCB-derived cells beyond hematopoietic progenitors and current applications in regenerative medicine.

standard heterogeneous MSC cultures. Furthermore, MSCs are distinct from hematopoietic cells and lack surface expression of typical hematopoietic markers including CD3, CD14, CD31, CD34, CD45, CD144, vascular endothelial growth factor (VEGF)-R1, and VEGF-R2. In some reports, UCB-derived MSCs can be distinguished from BM-derived MSCs by the surface expression of intercellular adhesion molecule 1 (ICAM-1), also known as CD54, which is expressed at higher levels on UCB-derived MSCs than it is on BM-derived MSCs [2,11]. The biological importance of increased ICAM-1 surface expression remains under active study. UCB-derived MSCs, however, retain multilineage differentiation potential towards osteogenic, adipogenic, and chondrogenic lineages [11-13]. Moreover, UCB-derived MSCs can be induced towards typical neuron-like morphology (long bipolar extensions and branching ends) when cultured in a neuron-inducing medium [14]. Differentiation into neural cells can be confirmed by expression of cytoskeletal proteins found in neurons and astrocytes like β−tubulin III and glial fibrillary acidic protein [12].

UCB-derived MSCs can also differentiate towards hepatocyte-like cells, acquiring a hepatocyte gene expression profile, including increased expression of albumin, alpha fetoprotein (AFP), cytokeratin (CK)-18, glutamine synthetase, and tyrosine aminotransferase. In addition, the newly formed hepatocyte-like cells display functional low-density lipoprotein incorporation capability [15]. Additional studies have reported muscle cell generation from UCB-derived MSCs, which remains under active investigation [16]. Skeletal myogenic differentiation can be demonstrated by mRNA expression of MyoD and myogenin, 2 important transcription factors in myogenic differentiation [17]. Differentiation of MSCs into functional cardiomyocytes with expression of N-cadherin and cardiac troponin has been inconsistent. In particular, UCB-derived MSCs may have broader applicability in regenerative therapy than MSCs derived from adult tissues, such as marrow or adipose tissue, because of the loss of differentiating potential that is associated with aging [9]. Taken together, UCB-derived MSCs represent a promising candidate for stem cell−based regenerative therapy for a broad range of damaged tissues (Figure 1).

Although MSCs from cord blood appear promising, MSCs can also be derived from other cord blood itself, the placenta, and other tissues, including bone, muscles, and adipose tissue. Although direct comparisons between different tissues sources have not been studied extensively, it is likely that MSCs derived from different tissues may have particular advantages in specific indications. Compared with UCB- and BM-derived MSCs, adipose tissue−derived MSCs did not prevent glioblastoma cell growth in 1 study [18], whereas adipose tissue−derived MSCs are promising candidates for inhibiting melanoma cell growth [19]. Moreover, muscle-derived MSCs appear particularly potent in the modulation of myoblast proliferation, supporting the use of tissue-specific MSCs for particular applications [20].

It has proven more difficult to expand MSCs from UCB than it is with marrow-derived MSCs, with an estimated frequency of only 1000 to 5000 MSCs in a typical UCB unit of

Table 1
Registered Clinical Trials Using UCB Cells as Regenerative Therapy or Immune Modulation: An Update from Iafolla et al. [28]

Clinical Area	Targeted Disorder	UCB-Derived Cell Type Used in the Trial		Source of Cord Blood Cells			Route of UCB Cells Administration		
		Total Mononuclear Cells	MSC	Autologous	Allogeneic	Not Specified	Intravenous	Directly in Injured Organ	Not Specified
Cardiovascular diseases* (4 studies)	Hypoplastic left heart syndrome, ischemic cardiomyopathy, idiopathic dilated cardiomyopathy	2	2	2	1	1	0	4	0
Neurodegenerative disorders† (19 studies)	Cerebral palsy and paralysis, global developmental delay, neonatal encephalopathy	6	2	2	4	2	2	1	5
	Acquired brain injury, ischemic stroke and encephalopathy, critical limb ischemia	5	1	1	4	1	1	1	4
	Injured spinal cord	1	0	0	1	0	0	1	0
	Autism spectrum disorder	2	0	2	0	0	1	0	1
	Alzheimer disease	0	2	0	2	0	1	1	0
Metabolic disorders‡ (18 studies)	Errors of metabolism-congenital/inherited	7	0	0	3	4	1	0	6
	Epidermolysis bullosa, mucopolysaccharidosis	4	0	0	0	4	0	0	4
	Diabetes: type 1 and type 2, diabetic foot ulcers	0	5	0	3	2	3	1	1
	Liver cirrhosis, ischemic-type biliary lesion	1	1	0	1	1	1	0	1
Immunologic disorders§ (7 studies)	Acquired hearing loss	2	1	2	1	0	3	0	0
	Bronchopulmonary dysplasia	0	2	0	2	0	0	2	0
	Systemic lupus erythematosus	1	0	0	1	0	1	0	0
	Steroid-refractory acute or chronic GVHD	0	1	0	1	0	1	0	0
Bone disorders‖ (6 studies)	Osteopetrosis, articular cartilage defects and injury	3	3	0	5	1	0	3	3
Other disorders¶ (2 studies)	Ovarian failure	0	1	0	1	0	0	0	1
	Solid malignant tumors	0	1	0	1	0	0	0	1

GVHD indicates graft-versus-host disease.
Data presented are the number of studies.
A total of 57 trials were identified from a total of 606 trials involving human umbilical cord blood transplantation, as of November 1, 2014.
Database source: ClinicalTrials.org registry.
* NCT01883076, NCT01445041, NCT01946048, NCT01219452.
† NCT01639404, NCT01486732, NCT02025972, NCT01988584, NCT01991145, NCT01885663, NCT00375908, NCT02236065, NCT01929434, NCT01769716, NCT02176317, NCT01638819, NCT02054208, NCT01471613, NCT02256618, NCT01019681, NCT01962233, NCT01884155, NCT01297218.
‡ NCT00950846, NCT00950846, NCT00668564, NCT00176904, NCT00920972, NCT00654433, NCT00383448, NCT00881556, NCT00478244, NCT01238328, NCT00176917, NCT01350219, NCT01954147, NCT02138331, NCT01216865, NCT01415726, NCT01942915, NCT0222389.
§ NCT01343394, NCT01673789, NCT02038972, NCT01297205, NCT01897987, NCT00684255, NCT01549665.
‖ NCT00775931, NCT00638820, NCT01087398, NCT01733186, NCT01041001, NCT01626677.
¶ NCT01742533, NCT0043676.

approximately 100 mL [21]. Despite this challenge, successful production of clinical grade MSCs from cord blood or from placental tissues has been accomplished by private and public cell manufacturing facilities [22]. Beyond technical limitations in UCB-derived MSC expansion, considerable preclinical research activity has occurred using UCB-derived MSCs to facilitate tissue repair in animal studies.

Rodent models of cardiovascular disease, peripheral vascular disease, and neurological injury have been studied extensively and appear promising in terms of improved functional recovery after UCB-derived MSCs transplantation [2]. The route of administration remains variable in these studies and has not been optimized for all types of tissue damage. For example, efficacy in cardiac repair was first observed after direct myocardial injection, whereas infusion through a central venous catheter or intracoronary infusion may be more clinically relevant but has been less studied. Moreover, local delivery of MSCs can be more challenging in particular sites such as the central nervous system. UCB-derived MSCs have also demonstrated promise in xenogeneic mouse models of liver injury [23] and islet regeneration in type 1 diabetes [24]. Preclinical studies in a rabbit model of intervertebral disc degeneration showed improvement using UCB-derived MSCs that could differentiate into chondrocyte-like cells [25]. Another study using larger animal models addressed the use of equine UCB-derived MSCs for immune modulation in horses [26].

The possibility that MSC-derived effects may be contained in extracellular microvesicles such as exosomes that are released from MSCs, and can integrate in target cells to reprogram of modify gene expression patterns was recently summarized in a systematic review [27]. Although significant publication bias is suspected, it appears that MSC-derived exosomes may have therapeutic benefit in tissue repair and/or immune modulation and may be another option to consider regarding the manufacturing of MSC-derived cellular products.

The number of patients treated in clinical trials using UCB-derived MSCs continues to increase. A recent systematic review published by Iafolla et al. summarizes the use of cord

Table 2
Ongoing clinical trials in regenerative medicine field using MSCs from diverse umbilical cord tissues.

	Wharton's Jelly-Derived MSCs, n	Placenta-Derived MSCs, n	Cord Blood–Derived MSCs, n
Cardiovascular diseases	2 NCT02368587 Ischemic cardiomyopathy NCT01291329 Acute myocardial infarction	0	2 NCT01946048 Ischemic Cardiomyopathy NCT01219452 Idiopathic Dilated Cardiomyopathy
Neurodegenerative disorders	0	0	5 NCT01929434 Cerebral Paralysis NCT02054208 Alzheimer's Disease NCT01962233 Ischemic Encephalopathy NCT01297218 Alzheimer's Disease NCT01884155 Stroke
Metabolic disorders	0	1 NCT01413035 Type 2 diabetes	6 NCT01350219 Type 1 diabetes NCT01954147 Type 1 diabetes NCT02138331 Type 1 diabetes NCT01216865 Diabetic foot NCT01415726 Type 2 diabetes NCT02223897 Ischemic-type biliary lesions
Immunologic disorders	0	1 NCT00749164 GVHD immunomudulation	4 NCT01673789 Alopecia areata NCT01297205 Bronchopulmonary dysplasia NCT01897987 Bronchopulmonary dysplasia NCT01549665 Steroid-refractory acute or chronic GVHD
Bone disorders	1 NCT01166776 Avascular necrosis	1 NCT01420432 Ankylosing spondylitis	3 NCT01733186 Articular cartilage defects NCT01041001 Knee articular cartilage injury NCT01626677 Knee articular cartilage injury
Other disorders	0	4 NCT01385644 Pulmonary fibrosis NCT02395029 Peyronie's disease NCT02398370 Erectile dysfunction NCT01649752 ICSI rate improvement	2 NCT01742533 Premature ovarian failure NCT00436761 Refractory or relapsed malignant solid tumors

blood cells, including MSCs, for novel indications in regenerative therapy and immune modulation [28]. The main indications for treating patients with cord blood–derived MSCs in these studies were cardiovascular disease, neurodegenerative disorders, and metabolic or immunologic disorders. In addition to published studies, ongoing registered clinical studies were also systematically summarized to provide an indication of the trend regarding new indications that may be expected in the near future. Table 1 provides a more recent update since Iafolla et al. [28] published the systematic review and attests that the use of UCB cells in human trials is increasing. From the number of registered trials, we should expect increasing activity using cord blood–derived MSCs to treat patients with neurological disorders and for immune modulation. Although some studies use MSCs expanded from UCB, many trials infuse unmanipulated or minimally manipulated total mononuclear cells from UCB units, making it more difficult to know which cell type is most responsible for the clinical benefit in these emerging indications. It will be difficult to conclude that MSCs are responsible for benefits observed in these patients and iterative improvements in the cell-based therapy strategies may be difficult unless cellular products are well characterized.

Despite the rapid growth in clinical studies of cord blood–derived stem and progenitor cells for an expanding list of new indications, surprisingly little is known about the mechanisms of cell-mediated tissue repair or immune modulation. In vivo monitoring of cell engraftment and survival has been used to gain greater insight regarding the capacity of cells to home and engraft in areas of tissue damage. The most popular technique to track UCB-derived MSCs in preclinical animal studies is based on the green fluorescent protein reporter gene transfection. After sacrificing treated animals and assessing injured tissues, such as the brain, using microscopic analysis, green fluorescent protein signals demonstrate that UCB stem cells can home to the injured brain [29]. In recipients of UCB transplantation, donor cells in the brain have been described at autopsy in patients with Hunter disease [30]. Live tracking in animals has recently emerged as a new and powerful approach to seeing how transplanted cells home to areas of tissue damage but has not yet been reported in patients. In 1 example, cord blood–derived progenitors were transduced with a retroviral symporter system and combined with positron emission tomography and magnetic resonance imaging to identify transplanted cells in the injured myocardium on day 7 after myocardial transplantation [31].

Homing of transplanted cells to areas of tissue damage is a key element of successful cell-based therapy. Building on knowledge of how hematopoietic progenitors home to BM via CXCR4-SDF-1 interactions and the PI3K/Akt pathway, many groups have provided insight on MSC homing to areas of ischemic tissue injury [32]. Indeed, MSCs appear to sense hypoxia and can migrate to areas of ischemia, thereby focusing chemoattractant gradients in the areas of damage [33]. Human MSCs mRNA expression and flow cytometry analysis have identified several cytokines/chemokine receptors that could be involved in MSC homing, including CCR1, CCR2, CCR4, CCR7, CCR9, as well as CXCR5 and CXCR6 [34,35]. All of these receptors respond to their cognate ligand by inducing human MSC migration in chemotactic transwell chambers. Trafficking of MSCs may also be influenced by molecules other than CC or CXC receptor ligands. Sackstein et al. demonstrated that MSCs use CD44 and that MSC homing can be improved by modifying the glycosylation pattern on CD44 [36]. This capacity to modulate homing of MSCs and to increase their homing function or ability to target specific tissues may prove very useful for future clinical applications. Moreover, the observation that infused MSCs may get trapped in the lung and induce favorable local repair responses [37] reopens the debate regarding direct injection of MSCs into injured organs. When MSCs or other specific progenitors localize in injured tissues, transmigration mechanisms from the bloodstream into the tissue appears critical but remains poorly understood. Both passive migration into tissues and active mechanisms similar to leukocyte transmigration have been described and strategies to enhance this process represent promising future strategies to enhance cell-based regenerative therapy. Phenomena such as transdifferentiation of MSCs and other progenitor cells to new cell types have been described in vitro, along with the observation that transplanted cells can fuse with resident cells in the injured tissue, such as the liver [21,38]. However, therapeutic benefit can also be observed without any significant functional engraftment of transplanted MSCs, suggesting the paracrine function of MSCs is central to their therapeutic effects.

It is important to acknowledge that most studies and ongoing human trials using umbilical cord–derived MSCs have used UCB itself rather than placental tissues or cord-related tissue. In parallel, an increasing number of studies describe the isolation and characterization of MSCs from placental tissue [39] or Wharton's jelly [40], with more robust and rapid expansion of cells. Some ongoing clinical trials also describe the use of umbilical cord matrix–derived MSCs (see Table 2). It remains premature to compare in vivo studies of tissue regeneration using MSCs derived from UCB compared with from other sources.

Cord blood–derived MSCs appear poised for increased clinical studies in the near future with many preclinical and clinical studies supporting their use. We anticipate ongoing refinement in cell manufacturing that will facilitate more widespread application of these promising strategies. Governments, health authorities, and blood establishments should prepare for increasing presence of cord blood MSC–based therapies in the near future.

ENDOTHELIAL-LIKE VASCULAR PROGENITOR CELLS

A number of cells with angiogenic capacity, collectively termed endothelial-like vascular progenitors, have been studied extensively in recent years and have the capacity to facilitate repair after vascular injury in animal models of myocardial injury, cerebrovascular damage, kidney injury, and other types of organ damage. Endothelial progenitor cells (EPCs) were first described in 1997 by Asahara et al. by isolating a subset of CD34-selected cells from BM that coexpressed surface marker CD133 and the endothelial lineage marker, KDR (VEGFR-2) [41]. Absence of CD45 discriminated EPCs from hematopoietic progenitors and CD34 antigen distinguishes EPCs from circulating mature endothelial cells. Additional cell types have been labeled as EPCs and include early outgrowth cells, circulating angiogenic cells that express Tie-2, and late outgrowth endothelial progenitors or endothelial colony-forming cells (ECFCs) [42]. Different EPC subtypes can contribute to vascular development and/or reconstitution to varying degrees. ECFCs, for example, have a high rate of proliferation and can integrate into damaged tissues in some animal studies of hind-limb ischemia when early passage cells are used, whereas circulating angiogenic cells are monocyte-derived cells that are isolated from peripheral blood and are short lived, can home to damaged tissues, and induce paracrine healing without tissue integration. The recruitment of EPCs to damaged tissues is facilitated by the elaboration of cytokines and other signals after hypoxic injury, inflammation-induced injury, radiation injury, or other modes of tissue damage. We recently demonstrated that endothelial cells produced increased levels of stromal cell–derived factor (SDF)-1α after lipopolysaccharide (LPS)-induced injury compared with hypoxic or radiation-associated injury. The migration of ECFCs, likewise, was mediated via distinct mechanisms with increased expression of E-selectin ligands on ECFCs induced by SDF-1α binding to CXCR4 after LPS-induced injury of endothelial cells [43]. The extent to which specific angiogenic cells or EPCs are mobilized into the peripheral circulation may provide a useful prognostic biomarker, with specific subsets of circulating CD34-positive angiogenic cells correlating with cardiovascular risk and the number of ECFCs correlating with Framingham risk scores [44,45].

ECFCs and circulating angiogenic cells can be readily expanded from UCB and peripheral blood, whereas angiogenic CD34-selected cells are more readily isolated from BM. Moreover, UCB-derived EPCs have been shown to engraft better after injection into ischemic tissues in experimental animal models compared with adult-derived EPCs [46]. In addition to facilitating rapid repair of ischemic injury in animal models, EPC-like cells can promote vascular re-endothelialization of injured arteries, as reported in 1 preclinical study involving arterial injury in mice [47]. UCB-derived EPCs have also been tested in the setting of enhanced efficacy of islet transplantation in a mouse model of diabetes [48] (Figure 1).

EPC-like cells and angiogenic cells are often impaired in patients with vascular disease, such as coronary artery disease, and in patients with diabetes, and strategies to augment the function of these cells have been addressed by several groups, building on insight regarding key mechanisms of action. We recently reported on the importance of T-cell acute lymphocytic leukemia protein-1 (TAL1) as a central transcription factor that regulates a complex gene network implicated in migration, adhesion, and tubule-forming functions of ECFC [49]. Moreover, TAL1 colocalizes with enzymes such as p300 (a histone acetylase) and various histone deacetylases and demethylases, underscoring the importance of epigenetic histone modification in the control of gene expression networks in ECFCs. Low-dose histone deacetylase (HDAC) inhibitors were shown to augment the vascular repair

function of ECFCs, including more rapid repair of peripheral vascular ischemia in mice. Others have also demonstrated the relevance of epigenetic histone and DNA modifiers in the control of EPC-like vascular repair function in animal models and this has been recently reviewed by Fraineau et al. [50]. Inhibition of apoptotic pathways such as PI3K/Akt using nicotine [51] and GSK-3B inhibitors also appear promising as a means of ex vivo priming of EPC-like cells before cell-based vascular therapy [47].

Fucosylation is another potential mechanism relevant in the promotion of EPC function. Overexpression of fucosyltransferase in EPCs enhance EPC homing function and neovascularization in a mouse model of hind-limb ischemia via E- and P-selectin expression improvement [52]. As well, angiotensin converting enzyme 2 priming and overexpression, by lentivirus transduction, improved UCB-derived EPC migration and tube formation through upregulation of endothelial nitric oxide synthase (eNOS) and Nox pathways [53]. An ongoing multicenter clinical trial of autologous use of early outgrowth EPCs transfected with eNOS continues to enroll patients (NCT00936819 Clinicaltrials.gov identifier).

Development of culture methods for manufacturing certain EPC populations remains a challenge and upscaling cell manufacturing and production methods remains a barrier for EPC-based clinical translation. Moon et al. proposed a xeno-free autologous culture system based on the use of UCB-derived serum extracts combined with UCB-derived collagen to coat plastic culture plates. UCB-derived EPCs cultured in this culture system promote limb salvage in a mouse model of hind-limb ischemia, suggesting that UCB represents a source of biomaterials [54]. Other developments are expected soon, such as serum-free media and the development of reagents suitable for human use and potency assays to assess cell product activity.

OTHER UCB-DERIVED CELLS WITH REGENERATIVE CAPABILITIES

Additional nonhematopoietic stem cell populations contained in UCB include unrestricted somatic stem cells (USSCs) [55]. In culture, USSCs exhibit similar morphology to UCB-derived MSCs although USSCs can be distinguished by greater DLK1 expression and reduced capacity for adipocyte differentiation [56]. Our understanding of potential developmental hierarchies of USSCs and MSCs derived from UCB continues to be refined and may shape future clinical applications in regenerative therapy.

Another MSC-like cell that can be isolated from UCB includes progenitors expressing high levels of aldehyde dehydrogenase. Aldehyde dehydrogenase–positive cells have an important regenerative capability in animal models of ischemic disease [57], myocardial infarction [58], and islet regeneration [59]. Additional work is needed before clinical trials can be envisioned.

Very small embryonic-like stem cells (VSELs) have also been described in UCB as pluripotent stem cells with significant regenerative potential. It is likely that VSELs are retained in the discarded red cell fraction after centrifugation-based methods of red cell depletion of UCB units. Moreover, pluripotent genes including Oct-4, Nanog, and Tert are expressed at high levels in VSELs [60]. Isolation of VSELs by UCB processing may offer great potential for regenerative therapy.

The use of induced pluripotent stem cells (iPSCs) remains an interesting avenue for future research. Wang et al. generated iPSCs from UCB mononuclear cells using a lentiviral-mediated gene transfer [61]. The newly reprogrammed cells express specific pluripotency markers (Oct4, Sox2, KLF4) at molecular and protein levels. Moreover, reprogrammed cells can acquire the capacity to differentiate into all the 3 germ layers [61]. UCB-derived iPSCs were also tested as a source of dopaminergic neurons in a preclinical rodent model of Parkinson's disease [62]. Beyond neural maturation, a reduced number of undifferentiated proliferating cells occurred after iPSC transplantation. This is reassuring regarding the potential risk associated with iPSC therapy regarding tumor development. To date, methods of introducing transcription factors required for cellular reprogramming of more mature cells within UCB involves gene transfer methods that are not ready for clinical application. Further optimization of nonintegrative methods to enhance gene expression may allow this technology to evolve towards clinical application [63,64] (Figure 1).

UCB may have additional utility in regenerative applications by using cord-derived noncellular components (serum) or platelet gels that are being tested in an ongoing clinical trial for treatment of diabetic foot ulcers (NCT02134132 ClinicalTrials.gov identifier).

TECHNICAL CONSIDERATIONS FOR NOVEL CORD BLOOD–DERIVED APPLICATIONS

Although both autologous and allogeneic cord blood units have been used for regenerative therapy and/or immune modulation (Table 1), more studies are needed to clarify which source of cells is preferred for specific uses in regenerative therapy. Moreover, implications related to banking cord blood in public and private settings are worth considering in the context of regenerative therapy. To optimize UCB unit utilization in regenerative medicine, integration with current cord blood banking establishments would be most cost effective. Current public UCB banking efforts are focused on HLA diversity and banking units with high cellular content that will facilitate timely hematopoietic engraftment in recipients undergoing hematopoietic stem cell transplantation (HSCT). The use of UCB in regenerative applications, however, may rely on units with distinct properties that may not be ideal for HSCT. If specific cell types are needed, such as MSCs or ECFCs, these cells may need to be expanded first and stored by banking establishments or alternatively, cryopreserved unmanipulated units could be requested by a cell manufacturing partner or manufactured using cell culture facilities at transplantation centers. Some types of cellular products are best prepared using fresh cells; however, while other applications may be feasible using thawed units. The selection of units for regenerative applications, however, could be limited to more common HLA haplotypes, preserving more precious UCB units from donors with rare HLA haplotypes for HSCT. Some applications of UCB-derived cell-based regenerative therapy may not require stringent HLA matching at all, such as the use of third-party MSC-like cells [65]. Recent reports suggest third-party ECFCs do not stimulate immune reactions and may be similar to MSCs, indicating that the use of third-party ECFCs may be possible [66]. This will require more study, however, to be assured that immune rejection of cells does not occur at significant levels. Likewise, units that contain high total nucleated cell counts should be reserved for HSCT applications, as the dose of cells required for regenerative therapy is less well defined. Greater understanding of specific biomarkers that characterize UCB units that are ideal for

regenerative therapy may allow the ability to distinguish these units from products that are more suitable for HSCT.

Cell manufacturing methods for the production of cord blood–derived products, such as MSCs, ECFCs, and other cell types, remain under active development. In particular, use of reagents approved for human use has stimulated the development of serum-free media, synthetic coating for plastic culture ware, and closed culture systems in highly regulated manufacturing facilities. Interestingly, cord blood itself represents a potential source of reagents to support the growth of particular cell products, such as MSCs, that can be expanded using allogeneic UCB serum. MSCs cultured in this manner displayed higher self-renewal and enhanced osteogenic potential in 1 study [67]. Moreover, autologous UCB serum supplementation also appears promising [68]. Another promising opportunity related to UCB-derived MSCs culture is the use of platelet lysate. Generated by repeated freeze and thaw cycles followed by membrane disruption, platelet lysate contains growth factors, attachment factors, microRNA, exosomes, and cytokines [69]. In parallel, UCB plasma has also been shown to support efficient expansion of UCB-derived EPCs [70].

Because ex vivo culture can lead to spontaneous cell transformation and uncontrolled cellular proliferation, it is important to evaluate the safety of expanded UCB cells before injecting into humans. Capelli et al. have proposed that genetic abnormalities be screened by karyotype and by more refined analysis of small genetic lesions. High resolution analysis, however, is resource intensive. *Sox2* and *Nanog* have recently been implicated in genetic signatures associated with malignant transformation and only nonsignificant levels of expression of these genes are normally observed in cultured UCB-derived MSCs [11]. Rigorous safety monitoring in preclinical animal trials and human studies will be critical. The use of early passage cells that have not undergone replicative senescence and remain at low risk for malignant transformation remains an encouraging approach.

UCB CELLS AS VEHICLE IN GENE THERAPY

The use of UCB-derived cells such as MSCs is a promising strategy for gene therapy or drug delivery (Figure 1). One major challenge in pharmacology is overcoming the blood-brain barrier to treat neurological disorders, including neurodegenerative diseases. The fact that certain cell types such as MSCs are recruited to injured sites, such as the central nervous system, provides an opportunity to embrace the homing and migration function of these cells as a means of gene or drug delivery. MSCs can be modified with most viral vectors while preserving their in vivo potential for use in gene therapy trials [71]. One example involved adenovirus vector-associated brain-derived neurotrophic factor delivery in an animal model of cerebral ischemia that was associated with significant functional improvement [72]. Neuroprotective effects can also be improved by gene-modified human MSCs expressing placental growth factor [73] or β1-integrin [74]. MSC-based gene therapy has also been investigated in models of immunomodulation in models of inflammation and cancer [75]. In an animal model of glioma, adenoviral-mediated transduction of UCB-derived MSCs to overexpress tumor necrosis factor-related apoptosis-inducing ligand lead to inhibition of tumor growth [76]. Engineering of UCB-derived MSCs to secret IL-12 also demonstrate enhanced migratory capacity, inhibition of tumor growth, and prolonged survival in mice with glioma. Beyond brain cancer, UCB-derived MSCs have been used in gene therapy approaches in lung cancer. Zhang et al. engineered human UCB-derived MSCs to efficiently deliver secretable IL-24. IL-24-delivered by MSCs inhibited growth of lung cancer cells by apoptosis and cycle arrest [77].

Strategies to improve hematopoietic stem cell transplantation have also described gene transfer into UCB-derived CD34-selected hematopoietic stem cells (HSCs). CXCR4 overexpression in UCB-derived CD34-selected cells can increase SDF-1–mediated homing and retention in the marrow and accelerate engraftment after transplantation in mice. Upscaling to human clinical trials remains hampered by potential safety issues linked to the immunogenicity of viral carriers. CXCR4 overexpression using the nonviral cationic liposome agent IBAfect (IBA, Goettingen, Germany), however, appears promising for improving the efficacy of HSCT [78].

UCB-derived hematopoietic $CD34^+$ cells may be useful as a vehicle for gene therapy in human immunodeficiency virus (HIV) infection. Li et al. conducted preclinical studies towards the treatment of HIV-1–infected infants with genetically "immunized" $CD34^+$ cells derived from UCB using anti–HIV-1 hairpin ribozyme genes [79]. UCB transplantation in HIV patients using cells from donors harboring CCR5 mutations has also been reported as a means of preventing HIV entry and possible cure for these patients [80]. Given the rarity of CCR5 mutation in the general population, combined with challenges of finding HLA-compatible units for UCB transplantation, the development of gene-editing tools to knockout CCR5 in UCB units may be something to consider for the future [81].

UCB CELLS SECRETOME AS AN ESSENTIAL ACCESSORY IN REGENERATIVE MEDICINE

Many of the chief benefits of cell-based vascular repair using MSCs and EPCs have been attributed to their paracrine actions (Figure 1). MSCs have been used in the context of HSCT where they have an established role in the marrow microenvironment to support hematopoiesis through direct cell-to-cell contact and/or production of growth factors, cytokines/chemokines, adhesion molecules, extracellular matrix proteins, hormones, and lipid mediators [82,83]. Successful clinical trials reveal that cotransplantation of third-party UCB-derived MSCs accelerates hematopoietic engraftment in patients undergoing unrelated UCB transplantation [65,84]. MSCs have also been used to rescue failed engraftment after autologous hematopoietic transplantation and likely function by providing secreted factors to restore hematopoietic niches [85]. Another clinical trial reported that ex vivo coculture of MSCs and HSCs was able to enhance HSC engraftment [86]. MSCs appear to prime HSCs by recapitulating some of the physiological cues of the stem cell niche that are missing in suspension culture and in conditioned transplant recipients.

UCB-derived MSCs also have immunomodulatory properties that can function much like BM-derived MSCs to inhibit allogeneic peripheral blood mononuclear cell proliferation and modulate dendritic cell–mediated T lymphocyte proliferation [87,88]. Immune modulation is facilitated by cell contact and soluble factors, as demonstrated in transwell systems. Soluble factors that modulate dendritic cell function continue to be characterized but include CD25 (IL2-R), CD38, and CD69 expression [89]. Prostaglandin E2 could also be implicated because prostaglandin E2 inhibition mitigates UCB-derived MSCs mediated immune modulation [90]. Natural killer cells cytotoxicity, known to accelerate immune responses, can also be impaired by molecules expressed by UCB-derived MSCs, including the gamma-secretase enzymes [91].

UCB-derived MSC immunomodulatory functions have been studied in various models of immunodegenerative disorders, including a neonatal rat model of hyperoxia-or pathogen-induced (*Escherichia coli*) lung injury [92], and type 2 diabetes. Indeed, a phase I/II clinical trial showed that UCB-derived multipotent progenitors that coexpress CD45 can educate autologous peripheral blood mononuclear cells in a closed loop system and can attenuate immunological regulators in type 2 diabetes. Insulin sensitivity was improved after treatment with some recovery of islet β cell function, perhaps through balancing of Th1/Th2/Th3 cytokine production [93].

Paracrine effects of MSCs in regenerative applications have also lead to the identification of secreted bioactive molecules. As an example, thrombospondin-2 secreted by human UCB-derived MSCs after administration to the synovial fluid in patients with osteoarthritis promoted chondrogenic differentiation. Knockdown of thrombospondin-2 expression on MSCs, using small interfering RNA, abolished the beneficial chondrogenic effects of MSCs [94].

Secretion of growth factors (VEGF, HGF, IGF, and TGF-β) and neutrophic factors (BDNF, β-NGF) by MSCs has been described in the context of nervous tissue regeneration through the activation and/or modulation of endogenous processes like the promotion of neurogenesis and angiogenesis. In vitro and in vivo experiments show that MSCs conditioned media is sufficient to allow the increase of neuronal survival in various neurological conditions (Parkinson's disease, spinal cord injury, ischemic stroke), and consequently, the improvement of animal behavior [95]. Although these observations were made from BM-derived MSCs, similar results would be expected with UCB-derived cells, given current promising results of clinical trials using UCB-derived MSCs. Considerable potential was also reported for MSC-secreted factors in regenerative processes associated to cardiovascular disease [83]. The use of conditioned media from cultured MSCs has lent further support to the important beneficial role of secreted factors, particularly in the field of cardiac repair but also in acute kidney injury [96].

MSCs cultured under hypoxic or anoxic conditions demonstrate a significant increase in the secretion of several angiogenic cytokines compared with that under normoxic conditions [97]. Molecular preconditioning of MSCs using bioactive proteins, such as TNF-α, SDF-1, TGF-α, or TNF-α, represent key signaling cues to increase production of VEGF in the conditioned medium compared with unstimulated MSCs [98]. Moreover, MSCs can also be engineered with transgenes for conditional gene expression with the aim of controlling the MSC secretome [83]. Analysis of the secretome of cultured MSCs must consider the effect of serum used for cell culture, which may contain many overlapping components and can interfere with detection. To circumvent this problem, MSCs can be cultured for a short time frame in serum-free medium or medium with defined serum replacements. Moreover, it is critical to consider that secretome expression in vitro is likely very different from what would be expected in vivo where cells can interact within different microenvironments and manifest unique secretome expression profiles.

Regenerative immunomodulation effects associated with UCB-derived stem cells are not limited to MSCs. Mouse acute lung injury, induced by lipopolysaccharide challenge, is reduced after transplantation of UCB derived $CD34^+$ cells. Comparatively, decreased inflammation (decreasing level of TNF-α, ICAM-1, IL-6, and iNOS mRNA levels) is associated with restoration of vascular integrity but is not observed after $CD34^-$ cells or PBS injection. These results suggest UCB-derived stem cells may have a potential role in the treatment of sepsis [99]. Ongoing human clinical trials are actively investigating this clinical area (NCT01849237 and NCT02421484, ClinicalTrials.gov identifiers).

Beyond endothelial recovery, EPCs were also shown to increase the production of osteogenic cells after coculture with human osteoblasts. This appears to involve a newly described paracrine effect related to the secretion of IL-1β from $CD34^+$ EPCs [100]. The paracrine effect of $CD133^+$ endothelial progenitors from UCB also appears to be associated with bioactive molecules contained in conditioned medium as well as to microvesicles released after 24 hours of culture. $CD133^+$ cell-derived microvesicles express mRNAs for several antiapoptotic and proangiopoietic factors, including kit ligand, IGF-1, VEGF, basic fibroblast growth factor, and IL-8 [101].

CONCLUSION

In summary, enthusiasm and momentum are increasing for the use of nonhematopoietic progenitor cells from human UCB for regenerative and immune modulatory treatments. MSC derived from cord blood have progressed to the point of clinical studies and routine clinical therapy appears imminent. Use of endothelial-like progenitors and other cell types continues to accelerate towards clinical application. The economics of cord blood banking and issues related to the cord blood bioeconomy will be continue to be considered in the coming years as cord blood banking efforts mature.

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