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Review

Neural Differentiation and Potential Use of Stem Cells From the Human Umbilical Cord for Central Nervous System Transplantation Therapy

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The human umbilical cord is a rich source of autologous stem and progenitor cells. Interestingly, subpopulations of these, particularly mesenchymal-like cells from both cord blood and the cord stroma, exhibited a potential to be differentiated into neuron-like cells in culture. Umbilical cord blood stem cells have demonstrated efficacy in reducing lesion sizes and enhancing behavioral recovery in animal models of ischemic and traumatic central nervous system (CNS) injury. Recent findings also suggest that neurons derived from cord stroma mesenchymal cells could alleviate movement disorders in hemiparkinsonian animal models. We review here the neurogenic potential of umbilical cord stem cells and discuss possibilities of their exploitation as an alternative to human embryonic stem cells or neural stem cells for transplantation therapy of traumatic CNS injury and neurodegenerative diseases.

Key words: human umbilical cord stem cells; neural progenitor cells; mesenchymal stem cells

Stem and progenitor cell-based transplantation therapies of human central nervous system (CNS) injuries/pathologies are promising therapeutic strategies for conditions ranging from ischemic injury to neurodegenerative diseases (for recent reviews see Goldman, 2005; Sonntag et al., 2005; Miller, 2006; Thuret et al., 2006; Garbuzova-Davis et al., 2006; Dunnett and Rosser, 2007). In theory, pluripotent embryonic stem (ES) cells are presumably the best choice in this regard. However, their potentials have been hampered by ethical objections (Gruen and Grabel, 2006) and a persistent lack of success in generating individual-specific ES cell lines by somatic cell nuclear transfer in primates (Hall et al., 2006). The embryonic and adult brain harbors various forms of adult neural stem cells and progenitor cells (Morshead and Van der Kooy, 2004; Emsley et al., 2005; Crain and Trainor, 2006; Ferretti et al., 2006; Sohur et al., 2006; Martino and Pluchino, 2006). These cells should be well suited for transplantation therapy and, being more committed to a neural lineage, have a higher potential of becoming functional neurons and glia and perhaps a lower probability of developing malignancy (Roy et al., 2006). However, it is unclear how patient-specific forms of these could be obtained by routine manipulations in a clinical setting.

Adult stem cells can be found in a variety of tissues. Some of these, such as those from skin (Joannides et al., 2004; Fernandes et al., 2006; Gingras et al., 2007) and adipose tissue (Safford et al., 2004; Ning et al., 2006), have demonstrated potential for neural differentiation. However, adult mesenchymal stem cells from bone marrow and umbilical cord (Sanchez-Ramos, 2002; Ortiz-Gonzales et al., 2004) that are capable of neural differentiation are probably the most realistic sources, in terms of clinical availability, for stem cell-based transplantation therapy of the CNS.

Adult mesenchymal stem cells are mesodermal in origin. The differentiation of these cells into bona fide neuronal or glial cells of neuroectodermal origin would involve differentiation across the germ layer boundaries. There is some evidence to suggest that this may be easier than expected, at least in vitro. Embryonic stem cells are known to assume a primitive neural progenitor fate when not under the influence of extrinsic factors (Smukler et al., 2006). Undifferentiated mesenchymal stem cells,

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depending on the source, have been known to be somewhat predisposed toward neural differentiation and intrinsically express some neuronal markers (Tondreau et al., 2004; Deng et al., 2006). The neural differentiation potential of adult bone marrow stromal cells (BMSCs) is perhaps the most extensively investigated. Although BMSCs are likely to contain a heterogenous mix of cells with multipotent differentiation capabilities, recent findings suggest that they could be coaxed toward neuronal differentiation in vitro, using procedures and reagents that are rather similar to isolation and differentiation of adult neural stem cells (Jiang et al., 2002; Hermann et al., 2004; Kondo et al., 2005; Bossolasco et al., 2005; Wislet-Gendebien et al., 2005, Tropel et al., 2006; Chen et al., 2006). On the other hand, evidence of BMSCs ''transdifferentiating'' into neural cells in vivo upon transplantation into animals has been more controversial (Mezey et al., 2000, 2003), with the neuronal identity of engrafted cells being attributed to their fusion with endogenous neurons (Weimann et al., 2003; Alvarez-Dolado et al., 2003; Rodic et al., 2004; Chen et al., 2006). The use of BMSCs in transplantation therapy of the CNS investigated in animal models has met with reasonable success (Dezawa, 2006; Tang et al., 2007; Bae et al., 2007), although clear functional integration of transplanted cells has yet to be conclusively demonstrated.

HUMAN UMBILICAL CORD AS A SOURCE OF STEM CELLS FOR TRANSPLANTATION THERAPY

The human umbilical cord has received attention as an alternative to bone marrow for patient-matched stem cell source because it can be noninvasively harvested at birth, and its quality is therefore not influenced by aging and postnatal viral infections. Stem cells have been isolated from different parts of the umbilical cord, which include cord blood (Lee et al., 2004; Sanberg et al., 2005; Garbuzova-Davis et al., 2006; Weiss and Troyer, 2006), subendothelial layer of cord vein (Romanov et al., 2003), cord vein endothelial lining (Romanov et al., 2003), and the cord matrix (or Wharton's jelly; Mitchell et al., 2003; Wang et al., 2004). As with BMSCs, there is ample evidence that umbilical cord blood and cord matrix mesenchymal stem cells could be differentiated into neural cells in vitro and in vivo (as discussed below). Furthermore, experimental transplantation in animal models of CNS disease (Garbuzova-Davis et al., 2003, 2005; Fu et al., 2006; Weiss et al., 2006; Lund et al., 2007) and injury (Kuh et al., 2005; Xiao et al., 2005; Dasari et al., 2007) have revealed some beneficial effects. In the paragraphs below, we summarize the reported attempts of neural differentiation by stem cells derived from the human umbilical cord blood and cord matrix (see Table I for a tabulated, concise summary). We then discuss the potential use of human umbilical cord-derived stem cells in CNS transplantation therapy in light of findings in animal models of ischemic/traumatic brain injury and neurodegenerative diseases.

CORD BLOOD STEM CELLS AND THEIR NEURAL DIFFERENTIATION IN VITRO AND IN VIVO

Umbilical cord blood is known to contain $CD34^+$ or $CD133^+$ hematopoietic stem cells, which can be used for bone marrow transplant (Rocha et al., 2004; Schoemans et al., 2006). Cord blood, however, also contains other types of multipotent stem cells, particularly $CD34^-$ and $CD45^-$ mesenchymal-like cells that could be isolated and propagated through adherent cultures. Other forms of progenitor cells, such as endothelial progenitors, have also been isolated from cord blood (Nagano et al., 2007). The neural differentiation potential of authentic hematopoietic stem cells from cord blood is tantalizing but is unclear at best. Jang et al. (2004) had reported some evidence of such a possibility. The authors showed that $CD133^+$ hematopoietic stem cells sorted out by flow cytometry could be induced to express neuronal, astrocytic, and oligodendroglial markers by retinoic acid. There also appeared to be an up-regulation of several transcription factors of the basic helix-loop-helix family (such as Pax6) that are important for early neurogenesis. The appearance of neuronal and glia markers is suggestive of some degree of transdifferentiation of these $CD133^+$ cells in culture. The extent of differentiation and functionality of the differentiated cells (for example, electrical excitability of the neurons-like cells) have, however, not been clarified.

Neural differentiation potential of cord blood-derived cells is more frequently associated with mesenchymal-like stem cells (Sanchez-Ramos et al., 2001; Goodwin et al., 2001; Ha et al., 2001; Buzanska et al., 2002; Jeong et al., 2004; Kögler et al., 2004; Sun et al., 2005) and is in many ways reminiscent of those from the bone marrow stroma (Chen et al., 2006). When mononuclear cells from cord blood are plated out onto a substratum followed by continuous culture, $CD34^-$ and $CD45^-$ could be selected by their growth as adherent cells. These cells could then be induced to exhibit varying degrees of neural-like differentiation using a combination of morphogens, such as retinoic acid, and growth factors, such as nerve growth factor (NGF), epidermal growth factor (EGF), and basic fibroblast growth factor (bFGF; see Table I). Morphological changes observed were generally accompanied by detectable elevations in both transcripts and proteins of neuronal markers, such as βIII-tubulin, and astroglia markers, such as glial fibrillary acidic protein (GFAP). Although a majority of the reported differentiation attempts did not include an assessment of the electrophysiological properties of the resulting neuron-like cells, these induced cells evidently expressed voltage and/or ligand-gated ion channels and exhibit outward and inward rectifying currents (Kögler et al., 2004; Sun et al., 2005).

A particularly well-characterized human umbilical cord blood-derived clonal stem cell line (HUCB-NSC) has been reported (Buzanska et al., 2006; Domanska-Janik et al., 2006). This was first isolated from nonhematopoietic clonogenic fractions that expressed the neural

TABLE I. Neurogenesis From Stem Cells of the Human Umbilical Cord*

Source of cells	Method of differentiation	Assessment of differentiation in vitro	Assessment of differentiation and/or function in vivo	Reference
Cord blood				
Mononuclear cells from cord blood	RA + NGF	Immunocytochemistry/ immunoblot for Musashi-1, βIII-tubulin, GFAP, and others	1. Transplantation into subventricular zone of neonatal rat brain; observed 20% survival after 1 month and cells with βIII-tubulin or GFAP labeling 2. Infusion into circulation of ALS model (G93A) mice; observed cell migration into parenchyma of brain and spinal cord with neural/glia gene expression; delay of ALS symptom onset	Sanchez-Ramos et al., 2001; Zigova et al., 2002; Garbuzova-Davis et al., 2003; Walczak et al., 2004
Clonogenic cells negatively selected for CD34 and CD45 from cord blood	1. RA, BDNF 2. Rat cortical neuron coculture	Immunocytochemistry for βIII-tubulin, GFAP and Gal-C		Buzanska et al., 2002
$CD45^-$ cord blood stem cells	bFGF and EGF	Immunocytochemistry/ immunoblot for βIII-tubulin, GFAP and Gal-C		Bicknese et al., 2002
$CD133^+$ hematopoietic stem cells	RA	Immunocytochemistry/ immunoblot/RT-PCR analysis of various neural and glia markers		Jang et al., 2004
Adherant cells derived from cord blood expressing nesenchymal markers	1. bFGF 2. DMSO/BHA/ forskolin	Immunocytochemistry/ immunoblot/RT-PCR analysis of various neural and glia markers		Jeong et al., 2004
$CD45^-$ ''unrestricted somatic stem cells'' from cord blood			Transplantation into hippocampal region of rats; observed tau positive cells with neuronal morphology surviving up to 3 months	Kögler et al., 2004
Cord blood cells			Transplantation with BDNF into injured spinal cord of rats; observed transplant cell survival, expression of neural/glia markers and improved behavioral scores	Kuh et al., 2005
Adherant cells derived from cord blood cells			Transplantation into the ventricles of embryonic day 16.5 rat embryos; observed no expression of neural/glia markers	Coenen et al., 2005
Cord blood-derived neural-like stem cell line (HUCB-NSC)	Neuromorphogens and cocultures	Expression of voltage and ligand gated channels		Sun et al., 2005; Jurga et al., 2006
Other parts of umbilical cord				
Wharton's jelly	1. bFGF 2. DMSO/BHA 3. Forskolin/VA/ HC/insulin (Woodbury's method)	Immunocytochemistry/ immunoblot for NSE, βIII-tubulin, neurofilament M and TH		Mitchell et al., 2003
Mesenchymal tissue of Wharton's jelly	Neuronal condition medium	1. Immunocytochemistry/ immunoblot for NeuN, neurofilament, GFAP, GAD, kainite receptor subunits etc. 2. Preliminary whole cell patch clamp		Fu et al., 2003

TABLE I continues on the next page

TABLE I. Neurogenesis From Stem Cells of the Human Umbilical Cord* (continued)

Source of cells	Method of differentiation	Assessment of differentiation in vitro	Assessment of differentiation and/or function in vivo	Reference
Wharton's jelly	*Salvia miltiorrhiza* extract and β-ME	Immunocytochemistry/RT-PCR for βIII-tubulin, neurofilament and GFAP		Ma et al., 2005
Umbilical cord perivascular cells (nonhematopoietic)	No neural differentiation			Sarugaser et al., 2005
Mesenchymal tissue of Wharton's jelly	1. bFGF/RA/β-ME 2. cAMP 3. HC/cAMP 4. aFGF/SHH/BDNF/NGF/vitronectin/IBMX/forskolin/PMA	Immunocytochemistry/immunoblot for NSE, GFAP		Lu et al., 2006
Mesenchymal tissue of Wharton's jelly	1. Neuronal conditioned medium 2. SHH/FGF8	1. Immunocytochemistry/immunoblot for TH, DBH and GAD 2. HPLC analysis of dopamine secretion	Striatal graft into 6-OHDA-lesioned rats 1. Immunofluorescence tracking of graft survival and migration using bis-benzamide and anti-human nuclear antigen 2. Attenuation of rotational behavior induced by amphetamine	Fu et al., 2006
Umbilical cord matrix	Woodbury's method	FACS analysis for nestin and TH; gene array profiling	Striatal graft into 6-OHDA-lesioned rats 1. Immunofluorescence tracking of graft survival and migration 2. Attenuation of rotational behavior induced by apomorphine 3. Effect of transplant on TH staining at transplanted sites	Weiss et al., 2006

*RA, retinoic acid; NGF, nerve growth factor; ALS, amyotrophic lateral sclerosis; BDNF, brain-derived neurotrophic factor; Gal-C, galactosylceramide; bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; DMSO, dimethylsulfoxide; BHA, butylated hydroxyanisole; VA, valproic acid; HC, hydrocortisone; NSE, neuronal specific enolase; TH, tyrosine hydroxylase; β-ME, β-mecaptoethanol; GFAP, glial fibrillary acidic protein; GAD, glutamate decarboxylase; SHH, sonic hedgehog; IBMX, 3-isobutyl-1-methylxanthine; PMA, phorbol myristate acetate; DBH, dopamine-β-hydroxylase; 6-OHDA, 6-hydroxydopamine.

stem cell marker nestin, had high self-renewal potency, and could be differentiated in vitro with neuromorphogens and/or cocultures with rat brain astrocytes or hippocampal slices. Differentiated cells expressed neuronal, astrocyte, and oligodendrocyte markers (Buzanska et al., 2002) and ion channels (Sun et al., 2005). Interestingly, continuous culture of this line under low-serum conditions enhanced its neuronal differentiation efficacy (Jurga et al., 2006).

Several attempts have also been made to examine the survival and fate of human umbilical cord blood stem cells transplanted into recipient rodent brains (Zigova et al., 2002; Walczak et al., 2004; Kögler et al., 2004; Coenan et al., 2005). The two earlier studies reported some degree of survival and observation of neuronal marker expression in cells transplanted into the neurogenic subventricular zone of neonatal, young, and adult rats. Kögler and colleagues documented the persistence, for up to 3 months, of tau-positive transplanted cells with typical neuronal morphology and migratory activity in adult rat hippocampus. However, Coenen et al. did not observe any neural or glial marker expression in $CD34^-/CD45^-$ human cord blood cells transplanted into the ventricles of E16.5 rat embryos. At the moment, the influence of pretreatment of cells (e.g., with or without induction of neuronal differentiation), the age of transplantation recipient, and the part of the brain into which cells are transplanted have not been

investigated in detail or in a systematic manner. Transplantation of cord blood-derived stem cells has, however, resulted in documented beneficial effects in rodent CNS disease models, as discussed below.

NEURAL DIFFERENTIATION BY STEM CELLS FROM THE HUMAN UMBILICAL CORD STROMA

Surrounding the two arteries and single vein of the umbilical cord is an extracellular matrix of proteoglycan-rich (Valiyaveettil et al., 2004) connective tissue stroma called *Wharton's jelly* (Takechi et al., 1993). Romanov et al. (2003) isolated endothelial and subendothelial cells from umbilical cord vein and reported the culturing of fibroblast-like cells. These do not have endothelial or lymphocyte antigens, but express smooth muscle α-actin as well as several mesenchymal cell markers. Interestingly, these cells appeared multipotent, at least in terms of adipogenesis and osteogenesis, but their neurogenic potential was not reported. Sarugaser et al. (2005) also documented the isolation of human umbilical cord perivascular cells that express α-actin and mesenchymal markers. These cells have osteogenic potentials, but the authors failed to observe any neural differentiation. Wang et al. (2004) worked rather specifically with cells from the Wharton's jelly and demonstrated the ability of the derived mesenchymal-like cells to differentiate into cardiomyocytes as well as their adipogenic and osteogenic potential. However, neural differentiation was also not reported.

On the other hand, Mitchell et al. (2003) isolated cells from the Wharton's jelly after removal of the veins and arteries and maintained these for more than 80 generations. When confluent cultures were treated with bFGF and other neuronal differentiation reagents according to a protocol by Woodbury et al. (2000), the authors observed neural differentiation in terms of both morphology and the expression of neuron-specific enolase, βIII-tubulin, neurofilament M, and tyrosine hydroxylase. Similar neural differentiation potential of Wharton's jelly-derived mesenchymal cells has been observed by several other groups using conditioned medium from a mix culture of young rat brain cells (Fu et al., 2004), β-mercaptoethanol, and an herbal extract from *Salvia miltiorrhiza* (Ma et al., 2005) or an elaborate multistep protocol with neuromophogens and growth factors (Lu et al., 2006). The umbilical cord stroma likely contains multiple types of cells with stem cell-like properties. In agreement with this notion, it has been shown recently that there are distinct subpopulations of umbilical cord stroma-derived cells with different capacity for neuronal differentiation (Karahuseyinoglu et al., 2007).

It also appeared to be possible to generate specific subtypes of neurons from mesenchymal cells derived from Wharton's jelly. In an elaborate extension of their earlier study, Fu et al. (2006) had shown that dopaminergic neurons could be generated by stepwise differentiation of umbilical cord mesenchymal cells initially in neuronal condition medium but with subsequent steps involving incubations with sonic hedgehog (Shh) and FGF8. This is evidenced by tyrosine hydroxylase staining and measurable dopamine secretion into the culture medium. Expression of dopaminergic neuron markers has also been observed in neurally differentiated bone marrow mesenchymal cells (Tatard et al., 2007) and cord blood cells (Fallahi-Sichani et al., 2007). These neurons have not been characterized in detail beyond examination of marker expression, and it is unclear whether they are anywhere near those generated from human embryonic stem cells (Roy et al., 2006) in terms of properties and functionality. The cells described by Fu et al. exhibited long-term survival and migration after transplantation into 6-hydroxydopamine-lesioned rat striatum. Transplantation of these cells also alleviated the hemiparkinsonian model symptom of amphetamine-induced rotation in these rats. It is interesting to note that, in this regard, neurons subjected to the additional Shh and FGF8 differentiation steps are significantly more efficacious than neurons differentiated by neuronal condition medium only, indicating that the dopaminergic properties of the former did make a therapeutic difference. Weiss et al. (2006), on the other hand, had shown that even transplantation of undifferentiated umbilical cord matrix cells could attenuate the rotational behavior. It would seem that umbilical cord matrix cells, particularly the mesenchymal stem cells, have tremendous neural differentiation potential in vitro and are not inferior to BMSCs in this regard. On the other hand, their differentiation in vivo may be as limited as that of BMSCs.

UMBILICAL CORD STEM CELLS TESTED IN ANIMAL MODELS OF STROKE AND TRAUMATIC CNS INJURY

Umbilical cord blood has been tested extensively for its possible beneficial effects in animal models of stroke and spinal cord injury (Newman et al., 2004; Sanberg et al., 2005; Garbuzova-Davis et al., 2006). Earlier studies indicate that human umbilical cord blood cells introduced simply by intravenous infusion into rats subjected to middle cerebral artery occlusion (MCAO) improved functional recovery (Chen et al., 2001). Interestingly, some of these human cord blood cells had entered the site of stroke and expressed neuronal and astroglial markers. It was later shown that the reduction in infarct volume and improvement in functional recovery are dose-dependent on the number of cells transplanted (Vendrame et al., 2004) and that even cord blood infusion at 48 hr after the ischemic episode help reduced secondary events of inflammation and apoptotic cell death (Newcomb et al., 2006). However, both the nature of the cells responsible within the heterogeneous cord blood population and the mechanisms underlying the beneficial effect observed were unclear. Willing et al. (2003a) observed that cord blood administration by intravenous infusion could produce more long-term

functional benefits compared with direct striatal transplantation. The same authors also showed that infusion of mobilized peripheral blood could also be functionally beneficial (Willing et al., 2003b). In fact, it was shown that administration of cord blood-derived $CD34^+$ hematopoietic cells could promote stroke recovery via enhancement of angiogenesis (Taguchi et al., 2004). Also, there appeared to be no need for transplanted cells themselves to infiltrate the lesion site or engraftment to the periinfarct region in order to exhibit a beneficial effect. These cells could apparently provide neuroprotection if their secreted factors could cross the blood–brain barrier (Borlongan et al., 2004) or alternatively via a reduction of neuroinflammation (Vendrame et al., 2005; Newcomb et al., 2006).

Could nonhematopoietic stem cells in cord blood contribute to alleviating the extent of acute injury and contribute to functional recovery after stroke? Some evidence for this notion is provided by Xiao et al. (2005). The authors injected intravenously a nonhematopoietic cell line derived from cord blood into stroke model animals and observed a reduction of infarct volume as well as improved behavioral recovery. Cells with human markers at the site of lesion were too scanty to suggest that the effects seen were due to cell replacement, but brains with cells infused exhibited greater collateral sprouting from the undamaged hemisphere. The greater functional recovery may therefore be attributed to trophic actions of the infused cells, enhancing reorganization of nerve fiber connections within the injured brain.

As in the case of stroke models, human umbilical cord blood either infused or transplanted into animal spinal cord injury models improved behavioral recovery (Saporta et al., 2003; Zhao et al., 2004; Kuh et al., 2005). Again, $CD34^+$ hematopoietic cell populations are effective in this regard (Zhao et al., 2004; Nishio et al., 2006). Although a small number of transplanted cells may be found to express neuronal or glial marker, there is no indication that transplanted cord blood cells could improve functional recovery through cell replacement. A more recent study suggests that oligodendrocyte-like cells differentiated from cord blood stem cells could enhance locomotor function recovery after moderate spinal cord injury by remyelination of injured axons (Dasari et al., 2007). In spite of the extensive reports outlined above, it is clear that some significant advances are needed to propel the use of cord blood stem cells from animal experiments to the clinic.

UMBILICAL CORD STEM CELLS TESTED IN ANIMAL MODELS OF NEURODEGENERATIVE DISEASES AND OTHER BRAIN DISORDERS

Cells from the umbilical cord have also been examined in animal models of neurodegenerative diseases as possible transplantation therapy options (Newman et al., 2004; Sanberg et al., 2005; Garbuzova-Davis et al., 2006). Preliminary evidence suggests that human umbilical cord blood infusion could potentially benefit animals modeling various neurodegenerative diseases (Ende and Chen, 2002). Human umbilical cord blood infusion into the Cu/Zn superoxide dismutase (SOD1) G93A mutant mouse model of amyotrophic lateral sclerosis have been shown to delay disease progression and death (Ende et al., 2000; Garbuzova-Davis et al., 2003). Transplanted human cells appeared to have migrated into a wide range of tissues, and some of those found at the brain parenchymal and spinal cord expressed neural and glial markers (Garbuzova-Davis et al., 2003).

The use of human umbilical cord blood in ameliorating symptoms of Parkinsonism in animal models has not been extensively investigated. However, as noted earlier, recent efforts with considerable positive indications have been reported with mesenchymal stem cells from the umbilical cord stroma (Fu et al., 2006; Weiss et al., 2006). This is reminiscent of the recent efforts in the use of mesenchymal cells derived from bone marrow stroma for transplantation into chemically lesioned rodent brain (Dezawa et al., 2004; Hellmann et al., 2006; Ye et al., 2007). These efforts may be driven in part by the apparent relative ease in deriving dopaminergic neuron types from stromal mesenchymal stem cells. One would expect to see umbilical cord stroma cells tested in primate models soon.

THE PROMISE OF HUMAN UMBILICAL CORD-DERIVED STEM CELLS IN THE TREATMENT OF NEURONAL DISEASES

The accounts described above suggest that the human umbilical cord is gaining interest as a practically useful source of stem cells for treating neuronal injury and diseases. In which ways are these cells able to match the much heralded embryonic stem cells, and are there specific niches of therapy that umbilical cord stem cells might fill?

At the very least, these cells could serve an autologous, patient-specific source for more ''passive'' modes of transplantation therapy. Cord blood stem cells have been explored clinically for therapeutic purposes in the hematopoietic and cardiac systems as well as in a variety of other organs (Brunstein and Wagner, 2006; Goldstein et al., 2006; McGuckin et al., 2006). These cells have clearly demonstrated benefits in animal models of CNS injury and stroke. The lack of evidence of substantial lesion site engraftment, survival, and neural differentiation suggests that the beneficial effects may be largely derived from a ''bystander'' function (Martino and Pluchino, 2006) of these cells, either through secretion of neurotrophic/survival factors or through suppression of inflammatory death. Instead of ambitious goals of functional neural or glial cell replacement via transplantation engraftment, optimizing the ability of transplanted (or infused) cord blood stem cells to contribute to the survival and regenerative capacity of CNS lesion sites is

perhaps a more achievable short-term goal. Neural stem cell-like progenitors of mesenchymal origin from the umbilical cord stroma could also serve this purpose. These may be more useful, in that their relatively higher abundance compared with those in cord blood would facilitate clonal propagation and storage. Optimization of such ''passive'' transplantation strategies would include assessment of dose response, coadministration with or transgenic expression of neurotrophic factors, and more importantly the monitoring of oncogenic transformation after transplantation.

As far as cell replacement therapy is concerned, a particularly hopeful aspect for the use of umbilical cord stem cells is as an autologous stem cell source for Parkinson's disease. As mentioned above, adult mesenchymal stem cells have emerged as a main rival for human embryonic stem cells in dopaminergic neuron replacement therapy. The efficacious use of embryonic stem cells-derived dopaminergic neurons in ameliorating disease symptoms in hemiparkinson animal models has been extensively documented (Kim et al., 2002; Roy et al., 2006). The finding that mesenchymal stem cells from Wharton's jelly could be differentiated with some efficiency to dopaminergic neurons is encouraging. The amount of doparminergic neurons generated from umbilical cord is evidently much lower (Fu et al., 2006), but these have not been augmented with protocols such as coculture with immortalized human midbrain astrocytes (Roy et al., 2006). Better doparminergic neuronal differentiation could also be potentially achieved by controlled transgenic expression of factors such as Nurr1 (Jankovic et al., 2005). There is much more to be explored in terms of doparminergic neuron differentiation, including investigations on how to generate those with properties approximating the A9 dopaminergic neuron subtypes at the substantia nigra that are preferentially lost during Parkinson's disease (Sonntag et al., 2005). Of course, umbilical cord-derived stem cells may be limited in their ability to be differentiated into specific neuronal subtypes compared with embryonic stem cells (Wichterle et al., 2002), but their full potential in this regard should be explored.

In view of the continuous uncertainties associated with the derivation and use of human embryonic stem cells (particularly those of autologous origin), there has been a sustained interest in the development of adult mesenchymal stem cells for replacement and regenerative therapies. In the near future, one can expect to see advances in both basic science investigations on neuronal differentiation of umbilical cord-derived stem cells in culture and in vivo and attempts to apply and optimize its therapeutic efficacies in clinical trials of neurotrauma and neural diseases. Methods of banking and preservation of umbilical cord blood cells for hematopoietic therapies are reasonably well established (see, e.g., Adami et al., 2005; Meyer et al., 2006). However, optimized protocols, particularly for the clonal expansion of stem cells in umbilical cord blood/stroma, and the subsequent cryopreservation have to be carefully developed.

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