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Therapeutic Potentials of Mesenchymal Stem Cells Derived from Human Umbilical Cord

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Abstract Human umbilical cord-derived mesenchymal stem cells (hUC-MSCs), isolated from discarded extra-embryonic tissue after birth, are promising candidate source of mesenchymal stem cells (MSCs). Apart from their prominent advantages in abundant supply, painless collection, and faster self-renewal, hUC-MSCs have shown the potencies to differentiate into a variety of cells of three germ layers (such as bone, cartilage, adipose, skeletal muscle, cardiomyocyte, endothelium, hepatocyte-like cluster, islet-like cluster, neuron, astrocyte and oligodendrocyte), to synthesize and secret a set of trophic factors and cytokines, to support the expansion and function of other cells (like hematopoietic stem cells, embryonic stem cells, natural killer cells, islet-like cell clusters, neurons and glial cells) , to migrate toward and home to pathological areas, and to be readily transfected with conventional methods. Two excellent previous reviews documenting the characteristics of this cell population with special emphasis on its niche, isolation, surface markers and primitive properties have been published recently. In this review, we will firstly give a brief introduction of this cell population, and subsequently dwell on the findings of differential capacities with emphasis on its therapeutic potentials.

Keywords Umbilical cord · Mesenchymal stem cell · Wharton's Jelly · Stromal cell · Differential potential · Neurological diseases

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Brief Introduction of Umbilical Cord and Resident Stem Cells

Human umbilical cord, a connecting tissue of extra-embryonic origin lying between the mother and fetus, consists of two arteries, one vein, inter-vessels connective tissue and umbilical epithelium. The connective tissue, also referred as Wharton's Jelly, is made up of sponge-like structure weaved by collagen fibers, proteoglycan and embedded stromal cells [1]. Early studies indicated that resident stromal cells were responsible not only for synthesis of matrix components and distension of the cord, but also for cellular communication and constriction. Accordingly, they were also referred as myofibroblasts [2, 3]. However, it was not until 2003 that Mitchell et al [4] reported their successful isolation of matrix cells from porcine and human umbilical cord by explants' culture and Romanov et al [5] reported their isolation of mesenchymal like cells from sub-endothelial layer of human umbilical cord vein respectively when the umbilical cord-derived MSCs began to attract extensively research. Two excellent reviews documenting the characteristics of this cell population with special emphasis on its niche, isolation, surface markers and primitive properties have been published recently [6, 7]. In the following, we will focus on the recent research on differential and therapeutic potentials of these cells. For convenience of description, we referred the varied types of human umbilical cord derived cells with mesenchymal-like characteristics to hUC-MSCs.

Advantages of hUC-MSCs Over Embryonic Stem Cells, Adult Stem Cells and Fetus Tissue Derived Stem Cells

The past decades have witnessed an explosion in the number of stem cell populations isolated from a variety of

embryonic, fetal and adult tissues. Highly self-renewal capacity and pluripotency of differentiating into derivatives of all germ layers in vitro and in vivo have made embryonic stem cells (ESCs) a leading candidate for tissue engineering research and regenerative medicine [8], such as in the treatment of Parkinson's disease[9], amyotrophic lateral sclerosis [10], spinal cord injury [11, 12], stroke [13], cardiac diseases [14], diabetes [15], hematopoietic diseases [16], liver diseases [17] and lung diseases [18]. However, in addition to ethical and political concerns, their clinical application is also severely limited by their lack of accessibility, technique difficulties in purification and manipulation as well as concerns for formation of teratoma. In contrast, adult stem cells (ASCs) which can be easily harvested from various tissues, such as skin [19], bone marrow [20] and adipose [21], might be used clinically to treat disorders of vulnerable vital organs with fewer concerns than ESCs. However, their application becomes less preferable when considering limited numbers, decreased growth and differential capacities with increasing age as well as invasive harvesting procedures [22].

Different from the extensive research on ESCs and ASCs, fetal stem cells have a comparatively recent history. Over the past years, two distinct sources, the fetus proper (including fetal bone marrow [23], lung [24], spleen, liver [25], pancreas [26] and peripheral blood [27]) and the supportive extra-embryonic structures (such as umbilical cord blood [28], umbilical cord [29], amniotic fluid (AF) [30], placenta [31] and amnion [32]), have both generated putative stem cells. In the past 5 years, MSCs derived from umbilical cord are of particular interests due to their advantages over embryonic and adult counterparts. To begin with, umbilical cord is routinely discarded at parturition, thus little ethical controversy attends the harvest of the resident stem cell populations compared with the ethical concerns that plague the isolation of ESCs. Secondly, the extracorporeal nature of this source facilitates isolation by eliminating the invasive and discomfort extraction procedures as well as patient risks that attend adult stem cell isolation. Most significantly, the comparatively large volume of umbilical cord and ease of physical manipulation theoretically increase the number of stem cells that can be extracted, which make it possible to get substantial number of cells in several passages without need of long term culture and extensive expansion ex vivo. Above all, based on the time of generation and sequestration during early developmental period of ontogenesis, the umbilical cord may endow resident stem cell populations with enhanced potency. In the following, we will summarize recent findings of hUC-MSCs with special emphasis on their plasticity and potential applications in the treatment of various diseases.

Differential Potentials into Adipogenic, Chondrogenic, Osteogenic Lineages

As the hUC-MSCs originated from extra-embryonic mesoderm, their differentiation capacities into adipogenic, chondrogenic, osteogenic lineages have been extensively studied [33–37]. A recent study compares the cell-mediated remodeling of three-dimensional collagen I/III gels during osteogenic differentiation of BM-MSCs and UC-MSCs showed that both types of MSC display all features needed for effective bone fracture healing [35]. Another investigation comparing the chondrogenic potential of hBM-MSCs and hUC-MSCs revealed that hUC-MSC group had three times as much collagen as the hBM-MSC group, which indicates the former may be a more desirable option for fibro-cartilage tissue engineering [36]. In addition, the successful transformation of human umbilical cord stroma-derived stem cells (HUCSCs) mature adipocytes implies their therapy for esthetic purposes [37].

Differential Potential into Neural Cells and Therapeutic Potentials in Neurological Diseases

In Vitro Differentiation into Neural Cells

Apart from the differentiation capacities into classical mesenchymal lineages, the differentiation potency of hUC-MSCs into neural lineage has attracted extensive attention. The pioneering study in the neuronal induction of hUC-MSCs was demonstrated by Mitchell et al. [4] using a relatively complex and multi-step neuronal induction procedure as previously defined by Woodbury et al. [38]. Similar results of our study confirmed these findings by comparing modified protocol of Woodbury et al. [38] and protocol of several neurotrophic factors [29]. In another experiment, hUC-MSCs began to express neuron-specific proteins and exhibit retraction of cell body, elaboration of processes, and clustering of cells after three days' induction with neuronal conditioned medium (NCM). Between the 9th and 12th days, glutamate invoking inward current was found in the transformed cells, which suggested that the induced cells differentiated into mature neurons in the post mitosis phase at this stage [39]. Interestingly, pre-induction with basic fibroblast growth factor (bFGF) followed by induction with salvia miltiorrhiza or β-mercaptoethanol also induced hUC-MSCs into nerve-like cells with round cell bodies and multiple neurite-like extensions which formed networks reminiscent of primary cultures of neurons. In addition, expression of nestin, β-tubulin III (TUJ-1), neurofilament (NF) and glial fibrillary acidic protein

(GFAP) was shown by immunohistochemistry and markedly enhanced gene expression of pleiotrophin, a neurite outgrowth-promoting protein, and nestin was detected [40]. Using a three-step neural induction protocol consists of bFGF, β-mercaptoethanol, neurotrophic factor-3 (NT-3), nerve growth factor(NGF) and brain-derived neurotrphic factor (BDNF) for 14 days, 60% of the neuroglial cells transformed from hUC-MSCs were stained positive for microtubule-associated protein (MAP-2) and 32% stained positive for GFAP. Moreover, some of them expressed TuJ-1, synaptophysin and γ-aminobutyric acid (GABA) [41]. Expression of neuronal markers, such as neuron-specific nuclear protein (NeuN) and MAP2, by induced hUC-MSCs were also observed by other investigators [42]. More specifically, Weiss et al. [43] observed that induction with protocol similar to Woodbury et al. [38] resulted in a lower number of cells expressing markers for early neural progenitors (i.e., nestin), however, a greater number of cells expressed a mature neural marker for catecholaminergic cells - tyrosine hydroxylase (TH). In addition, Fu et al. [44] also succeeded in transforming hUC-MSCs into dopaminergic neurons in vitro through stepwise culture in NCM, sonic hedgehog, and FGF-8. Although most of the studies documented the differential capacities of human umbilical cord vein derived MSCs into mesenchymal lineages, only two reports have demonstrated the neural differential potency of these cells [45, 46]

In Vitro Expression of Neurotrophic Factors and Related Factors

Apart from the differential potential into cells of neural lineage, hUC-MSCs produce significant amounts of trophic factors for dopamine neurons, such as glial-derived neurotrophic factor (GDNF) and fibroblast growth factor (FGF)-20 [43]. They also secrete significantly higher level of several other neurotrophic factors, such as interleukin-6 (IL-6), FGF-2 and BDNF in vitro when compared with human placenta-derived cells and adult dermal fibroblasts [47]. In addition, greater quantities of granulocyte colony-stimulating factors (G-CSF), vascular endothelial growth factor (VEGF) and GDNF were produced by hUC-MSCs than hBM-MSCs before, during, and one day after neuronal differentiation [46]. BDNF was also higher in hUC-MSCs than in hBM-MSCs before neuronal differentiation [46]. These diffusible factors may partially account for their neurotrophic effect in alleviating deficits of animal model of neurological diseases.

Experimental Application in Cerebral Ischemia

Ischemic stroke, characterized by rapid onset of neurological injury due to interruption of blood flow to the brain, is a medical emergency which can cause permanent neurological damage, complications and death if not promptly diagnosed and treated, leading to a huge social and economic burden. Several novel neuron-restorative approaches are under investigation for treatment of stroke. Recent experimental animal data support the safety and effectiveness of stem cell transplantation in enhancing neurological recovery from severe ischemic episode [48]. A variety of stem cells are under evaluation in search for optimal implant resource. Compared with ESCs, NSCs and umbilical cord blood cells, MSCs seem to be a readily accessible source due to their easily isolation and rapid expansion properties.

Instead of MSCs derived from bone marrow [49], Ding et al. [41] transplanted approximately 1×10^6 clonally expanded hUC-MSCs into the cortex of middle cerebral artery occlusion (MCAO) rat models and observed significantly improved neurological function as well as considerably increased cortical neuronal activity. The transplanted hUC-MSCs migrated towards the ischemic boundary zone and differentiated into glial, neuronal, doublecortin$^+$, CXCR4$^+$, and vascular endothelial cells [41]. In addition, hUC-MSCs transplantation promoted the formation of new vessels to increase local cortical blood flow in the ischemic hemisphere and significantly increased expression of neurotrophic factors [41]. The author believed that modulation by stem cell-derived macrophage/microglial interactions and increased β1-integrin expression might enhance the angiogenic architecture as well as plasticity of the ischemic brain after the implantation of hUC-MSCs [41]. After transplanted 6×10^5 hUC-MSCs isolated from the endothelial/subendothelial layers of cord vein into the damaged hemisphere of immunosuppressed ischemic stroke rats, Koh et al [46] also found improved neurobehavioral function, reduced infarct volume and increased nestin-positive endogenous stem cells in the hippocampus albeit a relatively small number of transplanted cells expressed detectable levels of neuron-specific markers. They postulated that improvement in behavioral function might be related to the neuroprotective effects of hUC-MSCs which resulted in increase of endogenous neurogenesis and reduction of infarct volume rather than the formation of new networks between host neurons and the implanted hUC-MSCs [46]. Similarly, other investigators observed that transplanted hUC-MSCs survived for at least 5 weeks in the ischemic brain, significantly reduced injury volume and neurological functional deficits of the subjected rats, widely incorporated into cerebral vasculature with partly differentiation into endothelial cells, and substantially increased vascular density in ipsilateral hemisphere of stroke. Thus, the mechanism underlying accelerated neurological functional recovery after hUC-MSCs transplantation may be mediated by angiogenesis [50].

Experimental Application in Intracerebral Hemorrhage

In exploration of therapeutic potential of hUC-MSCs in intracerebral hemorrhage, CM-DiI labeled cells were intracerebrally transplanted into intracerebral hemorrhage (ICH) rat models established by injection of bacterial collagenase VII. The results demonstrated that hUC-MSCs treatment significantly improved neurological function deficits, increased vascular density in the lesion and decreased injury volume. In addition, substantially reduced leukocytes infiltration, microglial activation, reactive oxygen species level and matrix metalloproteinase's production in peri-ICH area in cell-treated group were observed as compared with PBS control group [51]. In conclusion, intracerebral administration of hUC-MSCs could accelerate neurological function recovery of ICH rat. The underlying mechanism may ascribe to their ability to inhibit inflammation and promote angiogenesis.

Experimental Application in Spinal Cord Injury

Stem cell administration is a viable therapeutic strategy for spinal cord injury (SCI) as demonstrated by previous research on transplantation of ESCs [52], neural stem cells, olfactory ensheathing cells, MSCs [53], umbilical cord blood cells [54] into animal models of SCI. However, several significant obstacles must be overcome before this research translates to the clinic, of which include identifying the best source of stem cells, optimizing their characteristics prior to transplantation, reducing the risks of stem cell therapy, developing large-scale manufacturing technologies, and fulfilling regulatory considerations for government approval [55]. A promising research reported by Yang et al [56] provided evidence that transplantation of hUC-MSCs derived from Wharton's jelly was an effective strategy to promote the regeneration of corticospinal fibers and locomotor recovery after spinal cord transection in the rat. Several weeks after transplantation of hUC-MSCs into the lesion site of spinal cord injured rats, significant improvements in locomotion and fewer astrocytes in the lesion site as well as activated microglia in rostral and caudal stumps of the lesion were observed as compared with the control group [56]. This recovery process was also accompanied by increased numbers of regenerated axons in the corticospinal tract and neurofilament-positive fibers around the lesion site. In addition, transplanted hUC-MSCs survived as long as 16 weeks, migrated from the implantation site for about 1.5 mm in the caudal direction of the rostrocaudal axis, and produced large amounts of human neutrophil-activating protein-2, NT-3, bFGF, glucocorticoid induced tumor necrosis factor receptor, and VEGF receptor 3 in the host spinal cord, which may help spinal cord repair [56]. In conclusion, the author concluded that the mechanism underlying promotive effect on the regeneration of severed corticospinal axons after transplantation of hUC-MSCs was likely via release of more cytokines or growth factors from the undifferentiated stem cells rather than the differentiation of these cells into neuronal or glial cells. Another possible explanation is the implanted hUC-MSCs can modulate the activities of microglia and reactive astrocytes [56]. When Wharton's jelly cells-derived neurospheres were transplanted in combination with BDNF into transected spinal cord rats, very few grafted cells survived while greatly improved Basso, Beattie and Bresnahan (BBB) scores, significantly increased axonal regeneration and reduced cavitations were observed [57]. These results further support the idea that improvement in functional outcome is unlikely to be explained by cellular replacement; however, the axonal regeneration and neuroprotective action actived by the grafted cells are possible mechanisms [57].

Experimental Application in Parkinson Disease

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive loss of striatal dopaminergic function. To date, a variety of stem cells have been explored to find a promising therapy for this unsatisfactorily treated disease in clinical settings [58]. Fu et al [44] found that transplantation of hUC-MSCs into the striatum of Parkinsonian rats could partially correct the lesion-induced amphetamine-evoked rotation. The transplanted cells could survive at least 4 months in vivo as identified by positive TH staining and migrated for 1.4 mm both rostrally and caudally. After transplantation of approximately 1,000 undifferentiated hUC-MSCs into striatum of hemiparkinsonian rats without immune suppression, Weiss et al [43] observed amelioration in apomorphine-induced rotations in the pilot test without evidence of formation of brain tumors as well as frank host immune rejection response. More importantly, the TH-positive DA neurons in the substantia nigra (SN) and ventral tegmental area (VTA) showed a valid correlation between the number of cells and the number of apomorphine-induced rotations [43]. In conclusion, the behavioral recovery of PD model animals may contribute to rescue of the degenerating DA neurons in the SN and VTA [43].

Experimental Application in Retinal Disease

Photoreceptor degeneration, which may benefit from cell-based therapies, presents a leading cause of blindness in the developed world. A recent study [47] compared the efficacy of four human-derived cell types, including umbilical tissue-derived cells (hUTCs), placenta-derived cells (hPTCs), and bone marrow MSCs (hMSCs) and adult

dermal fibroblasts (hADFs), in preserving photoreceptor integrity and visual functions after injection into the subretinal space of rats in the progress of degeneration. Although both hUTCs and hMSCs significantly reduced the degree of functional deterioration compared with hPTCs and hADFs, the hUTCs gave large areas of photoreceptor rescue while hMSCs gave only localized rescue. Due to no evidence of cell differentiation into neurons was found, the author proposed that one likely explanation underlying the mechanism of rescuing photoreceptors and visual functions by hUTCs is that they serve as a source of neurotrophic factors, which may include IL-6 and BDNF. Therefore, hUTCs may become a potential cell source for therapy of retinal degenerative diseases.

Experimental Application in Brain Injury

In exploration of whether BDNF modified hUC-MSCs could promote stem cells differentiation into neurons and enhance neuromotor function after brain injury, the investigators administrated transfected hUC-MSCs into the edge of cerebral lesion of athymic mice brain injury models induced by hydraulic pressure percussion [59]. They observed that gene-modified hUC-MSCs could improve neurological function and increase neuron specific enolase (NSE)-positive cells while decrease GFAP-positive cells and number of apoptosis cells [59], which indicated their potential application in cerebral injury.

Differential Potential into Islet-Like Clusters and Therapeutic Potential in Type 1 Diabetes

Diabetes mellitus is a devastating metabolic disease that affected 150 million people worldwide by the year 2000 and estimated to double this number in 2025 [60]. While tight control of blood glucose achieved by conventional or intensive insulin treatment, self blood glucose monitoring, and patient education can significantly prevent the development and retard the progression of chronic complications, replacement of a patient's islets is the only treatment of type 1 diabetes that achieves an insulin-independent, constant normoglycaemic state and avoids hypoglycaemic episodes [61]. However, islet transplantation has been hampered by the worldwide shortage of transplant-ready islets, immune rejection and recurrent attacks against islets by the underlying autoimmunity [62]. Currently, ESCs [63], pancreatic stem cells [64], hepatic stem cells [65], neural progenitor cells [66], bone marrow-derived cells [67], or umbilical cord blood cells [68] are under investigation for transplantable insulin-producing cells. However, both ESCs and fetus-derived stem cells have ethical problems which impede their application into the clinical settings. Meanwhile, bone marrow-derived cells were reported to have more limited expansion and differentiation capacities with advanced age [22] while the application of umbilical cord blood is always at the expense of hematopoietic stem cells albeit of other disadvantages.

A recently published research shows that hUC-MSCs derived from Wharton's Jelly may serve as a promising alternative cell source of transplantable islet-like clusters [69]. The investigators successfully induced hUC-MSCs into islet-like cell clusters in vitro through a four stage differentiation protocol in NCM and then transplanted them into the liver of streptozotocin-induced diabetic rats via laparotomy. These induced islet-like cell clusters were shown to contain human C-peptide and release human insulin in response to physiological glucose levels as well as express insulin and other pancreatic beta-cell-related genes. Accordingly, the hyperglycemia and glucose intolerance in diabetic rats were significantly alleviated after xenotransplantation of these clusters. Furthermore, the normalized stable blood glucose levels were maintained for over 9 weeks, without use of immunosuppressant. In addition to the existence of islet-like cell clusters in the liver of transplanted rats, some special fused liver cells were also found [69]. When considering their advantages such as large potential donor pool, rapid availability, no risk of discomfort for the donor, and low risk of rejection, hUC-MSCs may have the potential to become an excellent candidate in β-cell replacement therapy of diabetes.

Differential Potential into Hepatocyte-Like Cells and Therapeutic Potential in Hepatic Diseases

Liver cell transplantation is an emerging technique next to whole organ transplantation for the treatment of metabolic liver disease, liver fibrosis, and a variety of other end stage liver diseases [70]. Recent developments in stem cell technology have paved way for identifying novel candidate sources of liver cells to be used for regenerative purpose. Instead of ESCs with ethical debates and safety concern, fetal or adult liver cells encumbered by organ availability, BM-MSCs plagued with decreased expansion and differentiation capacities in advanced ages [22], Campard et al [71] observed that in vitro expanded UC-MSCs constitutively expressed markers of hepatic lineage and genes of enzymes involved in hepatic metabolism. After three steps of full term hepatogenic induction, differentiated UC-MSCs exhibited hepatocyte-like morphology, upregulated several hepatic markers, stored glycogen, produced urea, and exhibited an inducible CYP 3A4 activity. However, absence of some hepatic markers in differentiated UC-MSCs, such as HepPar1 or HNF-4, implied that their differentiation did not reach the level of mature hepatocytes [71]. More

recently, a simple one-step induction protocol with hepatocytic growth factor (HGF) and fibroblast growth factor-4 (FGF-4) by Zhang et al [72] were also proved to be effective in transforming hUC-MSCs into hepatocyte-like cells expressing the hepatocyte-specific markers albumin (ALB), human α-fetoprotein (AFP) and cytokeratin 18 (CK-18) [72]. Further studies showed that differentiated hUC-MSC could store glycogen and uptake low-density lipoprotein (LDL), which supported that they might serve as a favorable cell source for tissue engineering in the treatment of liver disease [72]. In addition, the differentiated functional hepatocyte-like cells may still retain their low immunogenicity in vitro [73], which facilitate the allotransplantation to replace the diseased liver cells.

In vivo investigations showed that, several weeks following injection into the spleen of SCID mice with partial hepatectomy, engrated hUC-MSCs were observed to express human albumin and AFP in recipient liver parenchyma and perivascular, which indicated they might become an alternative source for liver-directed cell therapies [71]. Several weeks after transplantation into the lesion livers of carbon tetrachloride (CCl4)-induced liver fibrosis rats, significant reduction in liver fibrosis with significantly lower levels of serum glutamic oxaloacetic transaminase, glutamic pyruvate transaminase, alpha-smooth muscle actin, and transforming growth factor-beta1 in the liver was observed. In addition, up-regulated expression of hepatic mesenchymal epithelial transition factor-phosphorylated type (Met-P) and hepatocyte growth factor was also found [74]. The engrafted hUC-MSCs did not differentiate into hepatocytes expressing human albumin or alpha-fetoprotein but secrete a variety of bioactive cytokines, including human cutaneous T cell-attracting chemokine, leukemia inhibitory factor, and prolactin, which may benefit the restoration of liver function and promotion of regeneration [74]. In similar CCl (4)-injured mouse hepatic injury, hUC-MSCs were found to express tryptophan 2, 3-dioxygenase, human alpha-fetoprotein, cytokeratin-18, fibroblast secretory protein-1 and alpha-smooth- muscle-actin after administration into the diseased livers. In addition, transplanted hUC-MSCs could reduce hepatocyte denaturation, inhibit hepatocyte apoptosis, decrease serum aminotransferases, and facilitate hepatocyte proliferation [75].

Differential Potential into Cardiomyocytes and Therapeutic Potential in Cardiovascular Diseases

The dynamics of stem cell research have been driven particularly by application in regenerative medicine with focus on various tissues and organs, among which include the cardiovascular system. Kadner et al [76] initially investigated the feasibility of using the umbilical cord cells (UCCs) as an alternative autologous cell source for cardiovascular tissue engineering by seeding UCCs on polymers to construct a tissue-engineered value. Further comparative study revealed that cells isolated from umbilical cord artery (UCA), vein (UCV), the whole umbilical cord (UCC) and saphenous vein segments (VC) have comparable cell growth, morphology, characteristics and tissue formation [77]. In addition, Hoerstrup et al [78] successfully constructed living, autologous pulmonary artery conduits tissue with human umbilical cord cells. More recently, a research group engineered living patches with human umbilical cord derived fibroblasts and human umbilical cord blood derived endothelial progenitor cells [79], while another group published their successful attempt in tissue engineering of autologous human heart valves using cryopreserved vascular umbilical cord cells [80]. Considering their excellent growth properties and tissue formation with biomechanical properties approaching native tissue in vitro, it seems that UCC represent a promising alternative autologous cell source for cardiovascular tissue engineering, offering the additional benefits of using juvenile cells and avoiding the invasive harvesting of intact vascular structures [81].

5-azacytidine, a chemical analogue of the cytosine nucleoside in the DNA and RNA helix, is currently used as a key chemical initiator of myogenic differentiation. Although some investigators succeeded in transformation Wharton's Jelly derived hUC-MSCs into cardiomyocytes by 5-azacytidine [33, 82] or cardiomyocyte- conditioned medium [33, 82] and observed a slight spontaneous beating [82], others failed to generate cardiomyocyte-like cells from hUC-MSCs, either spontaneously or after treatment with 5-azacytidine [83]. Further studies are needed to solve the discrepancy between the two studies. However, another group reported that MSCs isolated from endothelial/subendothelial layers of the human umbilical cord veins also had the potential of transdifferentiating into cardiomyocyte-like cells with typical ultrastructure and sarcomers as well as expression of several cardiac-specific genes [45]. Taken together, these observations indicate that hUC-MSCs can be chemically transformed into cardiomyocytes and considered as a source of cells for cellular cardiomyoplasty.

An in vivo study to investigate the therapeutic potential of hUC-MSCs in a rat myocardial infarction model revealed significantly improved cardiac function, markedly increased capillary and arteriole density, and notably decreased apoptotic cells in hUC-MSCs transplantation group compared with control group. In addition, some of transplanted cells survived in the infarcted myocardium, accumulated around arterioles and scattered in capillary networks [84]. Importantly, some of the cells expressed cardiac troponin-T, von Willebrand factor, and smooth muscle actin, indicating regeneration of damaged myocardium by cardiomyocytic,

endothelial, and smooth muscle differentiation of hUC-MSCs in the infarcted myocardium [84].

Taken together, these findings, both in vitro and in vivo, demonstrate that hUC-MSCs can be used to produce cardiomyocytes, construct artery conduits, engineer living patches and heart valves, and provide benefit in cardiac function recovery after acute myocardial infarction. Therefore, hUC-MSCs may become a promising cell source both for cardiovascular tissue engineering and for cellular cardiomyoplasty.

Differential Potential into Skeletal Muscles and Therapeutic Potential in Myogenic Diseases

To date, only one report has documented successful transformation of a subset of CD105(+)/CD31(-)/KDR(-) cells from Wharton's Jelly of umbilical cord into elongated, multinucleated cells expressing of Myf5 and MyoD in vitro [85]. Two weeks after injection into the tibialis anterior muscle of rats previously damaged with bupivacaine, these cells transformed into skeletal muscle cells as demonstrated by the co-localization of HLA-1 and sarcomeric tropomyosine antigens [85]. These observations provide the first demonstration that CD105 (+)/ CD31 (-)/KDR (-) cells are able to differentiate in vivo towards the myogenic lineage and benefit muscle regenerative process.

Differential Potential into Endothelial Cells and Therapeutic Potential in Angiogenesis and Re-Endothelialization of Engineered Tissue Grafts

Due to the number of endothelial progenitor cells (EPCs) obtained from adult bone marrow, cord blood and peripheral blood is limited, investigators began to search other cell sources to treat cardiovascular diseases. By culturing in an endothelial differentiation medium containing VEGF and bFGF after a proper period, Wu et al [84] found that hUC-MSCs differentiated into endothelial cells as demonstrated by acetylated low-density lipoprotein incorporation and expression of endothelial-specific proteins, such as platelet/endothelial cell adhesion molecule (PECAM) and CD34. After transplantation into ischemic mouse model, these cells sprouted from local injection point and differentiated into endothelial cells in the lesion hind limb [84]. In comparison of endothelial differentiation of hUC-MSCs and BM-MSCs, some investigators also found that hUC-MSCs had higher proliferate potential, higher expression of the endothelial-specific markers after induction and significantly higher total tubule length, diameter, and area in angiogenesis assay than those of differentiated BM-MSCs [86]. More recently, UC-MSCs transplanted into wire-injured femoral arteries in mice were shown to play a crucial role in reestablishing endothelial integrity in injured vessels by inhibiting neointimal hyperplasia [87]. Therefore, the cell population with characteristics of EPCs in human umbilical cord makes it a novel source of stem cells for therapeutic angiogenesis and re-endothelialization of engineered tissue grafts. Comparison between UC-MSCs and BM-MSCs suggests the former is a more favorable choice than the latter for neovascularization of engineered tissues.

Stromal Supporting Property

Supporting the Engraftment and Expansion of Haematopoietic Stem Cells

MSCs have been shown to facilitate haematopoietic stem cell (HSCs) growth in vitro [88] and in vivo [89]. One of the mechanistic basis for MSCs support of engraftment and expansion of donor HSCs is they constitutively secrete haematopoietic cytokines, such as IL-6, IL-7, IL-8, IL-11, IL-14, IL-15, macrophage colony-stimulating factor (M-CSF), flt-3 ligand and stem cell factor (SCF). In the context that stromal supporting property is one of the basic characteristics shared by MSCs of different origin, a recent investigation demonstrated that hUC-MSC shared a cytokine spectrum very similar to that of BM-MSCs, including expression of the mRNA of SCF, leukemia inhibitor factor (LIF), M-CSF, Flt3-ligand, IL-6, VEGF and stromal-derived factor-1 [90]. However, hUC-MCSs additionally expressed mRNA of granulocyte macrophage colony-stimulating factors (GM-CSF) and G-CSF [90]. After co-culture with $CD34^+$ cord blood cells for 5 weeks, no significant difference in colony-forming cells was observed between the $CD34^+$ cells/UC-MSC and $CD34^+$ cells/BM-MSC co-cultures, which indicated that UC was an excellent alternative to BM as a source of MSCs for cell therapies [90]. Bakhshi et al [91] also reported that hUC-MSCs, similar to their counterparts in bone marrow, could effectively support the growth of $CD34^+$ cord blood cells as demonstrated by long-term culture-initiating cell assays. Similarly, another group observed that UC-MSCs could produce significant higher concentrations of hematopoietic growth factors (including G-CSF, GM-CSF, HGF, LIF, IL-1a, IL-6, IL-8, and IL-11) than those of BM-MSCs in culture, augment hematopoietic colony formation when co-cultured with umbilical cord blood (UCB) mononuclear cells in vitro, and promote engraftment of human UCB cells or CD34(+) selected cells in mice when co-transplanted [92]. hUC-MSCs also efficiently increased homing and improved migration efficiency of UCB $CD34^+$ cells to bone marrow and spleen in vivo by expressing a high level of homing adhesion molecules,

including CD49e, CD31, CD62L, and CD11a [93]. However, a more recent comparative investigation showed that UC-MSCs are able to support long-term hematopoiesis in vitro, but their hematopoietic supportive capacity is weaker than those of BM-MSCs [94].

Maintaining the Survival and Function of Islet-Like Cell Clusters

In addition to the above mentioned successful attempt in deriving islet-like clusters from hUC-MSCs and application in control type 1 diabetes [95], there are also documents reported that hUC-MSCs had the potential of maintaining survival and function of islet-like cell clusters (ICCs) while increasing cell number and insulin secretion. Cytokine protein array revealed a twofold increase in levels of several cytokines, including IL-6, tissue inhibitor of metalloproteinases-1, tissue inhibitor of metalloproteinases-2, monocyte chemoattractant protein-1, growth related oncogene, HGF, insulin-like growth factor binding proteins 4, and IL-8 in coculture medium of hUC-MSCs [95]. These findings suggest that, in addition to serving as a promising alternative cell source for islet-like clusters, hUC-MSCs may also be employed in a novel culture approach to maintain islet cell survival and function before transplantation due to their significant potential to protect ICCs from damage during culture.

Supporting the Expansion of Natural Killer Cells

Natural killer (NK) cell-mediated cytotoxicity can control leukemia relapse while protecting patients from graft-versus-host disease (GVHD) after allogeneic stem cell transplant. Hence, it is attractive to expand and potentially utilize these cells for adoptive immunotherapy. After plating CD3-depleted UCB mononuclear cells with a feeder layer of irradiated hUC-MSCs, Boissel et al. [96] observed a significantly higher expansion of CD56(+)/CD3(-) NK cells in the presence of feeder layer in comparison without feeder layer. He proposed that UCB NK cell progenitors could be expanded to obtain large numbers and maintain an elevated cytotoxic profile by an irradiated feeder of hUC-MSCs [96].

Supporting the Expansion of ESCs

Clinical application of human ESCs requires the establishment of methods for their culture, either in the presence or absence of human-derived feeder cells [97]. In exploration of ability of non-immortalized cultured cells derived from human umbilical cord (HUC) to support ES cell culture, a primate ES cell line established and maintained with mouse embryonic fibroblasts was cultured on HUC cells for >3 months (HUC-maintained ES cells). These cells retained their expression of alkaline phosphatase, SSEA-4, Oct-3/4, and to a lesser extent Nanog, but did not express Rex-1 [98]. Nevertheless, HUC-maintained ES cells could produce ectoderm-, mesoderm- and endoderm-derived cells in teratoma that they formed in immunodeficient mice [98]. HUC-maintained ES cells also gave rise to hematopoietic cells, although this ability of HUC cells varied among HUC cell populations derived from different neonates [98]. In conclusion, HUC cells are promising as human material with which to maintain ES cells in a state that retains their ability to produce mature cells, including hematopoietic cells.

Supporting the Viability and Proliferation of Neural Cells

In addition to their differential potential into neural cells, conditioned media of hUC-MSCs was found to increase numbers of GFAP positive cells and O4 positive cells in glial cell cultures and increase numbers of MAP-2 positive cells in neurons cultures [99]. As for the mechanism, the hUC-MSCs most probably exert their effect on viability and proliferation of post-natal hippocampal cells via releasing neuroregulatory factors.

Immunosuppressive and Immunomodulatory Properties

MSCs, evoked only minimal immune reactivity, may have immunosuppressive and immunomodulatory effects [100]. In characterizing the immune properties of hUC-MSCs, some investigators observed that these cells inhibited splenocyte proliferation response to concanavalin A stimulation and proliferation of stimulated T cells in a two-way mixed lymphocyte reaction (MLR), however, they did neither inhibit nonstimulated splenocyte proliferation nor stimulate T-cell proliferation in a one-way MLR [101]. They also express molecules implicated in the immune modulation, such as VEGF, IL-6, but did not express costimulatory surface antigens, like CD40, CD80, and CD86 [101]. A recent comparative analysis of the immunomodulatory properties and immunosuppressive effect of MSCs derived from adult human tissues including bone marrow, adipose tissues, umbilical cord blood, and cord Wharton's jelly showed that there were no significant difference in levels of secreted factors from non-stimulated MSCs as well as phytohemagglutinin-induced T-cell proliferation [102]. However, Cho et al [103] found that hUC-MSCs could be activated to express MHCII and increase MHCI with IFN-γ stimulation in vitro although they were MHCI dull and negative for MHCII. Of great significance, hUC-MSCs did elicit an immune response when injected in an inflamed region or injected repeatedly in the same region or stimulated with IFN-γ prior to injection albeit a single injection of MHC-mismatched unactivated hUC-MSCs did not induce a detectable immune response. As clinical cellular repair

strategies may involve injection of allogeneic cells into inflamed regions of damaged tissue or repeated doses of cells to achieve desired benefit, these results on the immunogenicity of these cells in such circumstances may have important implications for optimal success and functional improvement for this cellular treatment strategy for diseased tissues.

Gene Modification

MSCs are amenable to be genetically modified, which makes them become a promising platform for cell and gene therapy and broadens their potential therapeutic applications in several fields, including improvement of stem cell engrafting and acceleration of hematopoietic reconstitution, treatment of severe graft-versus-host disease, utilization in targeting tumors and delivering anti-cancer molecules as well as cellular vehicle for protein-supplement gene therapy [104, 105]. Accordingly, the ability to introduce exogenous DNA into hUC-MSCs using conventional methods [106] renders them promising MSC candidates for gene therapies. The site of active tumorigenesis favoring home of exogenous MSCs further supports the rationale for developing engineered MSCs as a tool to track malignant tissues and deliver anticancer agents within the tumor microenvironment. Administration of hUC-MSCs expressing interferon-β with [107] or without 5-fluorouracil [108] was found to target to experimentally developed lung tumors and significantly reduce the tumor burden. hUC-MSCs modified to express BDNF were also found to improve neurological function and increase NSE-positive cells while decrease GFAP-positive cells and number of apoptosis cells after being administrated into the edge of lesion in athymic mice brain injury model induced by hydraulic pressure percussion [59]. In addition, hUC-MSCs can be genetically modified to express biologically active human factor IX and serve as an efficient drug delivery vehicle for somatic gene therapy of hemophilia B [109]. Whether these cells, like their counterparts of NSCs or MSCs from other resources [110], can target intractable cerebral glioma and serve as a vector for gene therapy with high specificity is under investigation in our laboratory.

Conclusion

Comparing with counterparts of other origins, hUC-MSCs have the following advantages: (1)The access of hUC-MSCs was not encumbered with ethical and legal constraints plaguing ESCs in that umbilical cord is an abandoned extra-embryonic tissue afterbirth; (2) Comparing with BM-MSCs which decreased in cell number, expansion ability and differentiation capacities with advanced age [22, 111–113], hUC-MSCs have higher number and stronger proliferation ability, which makes it possible to obtain substantial number of cells required for clinical transplantation in a short time [114]; (3) Comparing with harvesting success rate of MSCs from UCB as low as 6%, hUC-MSCs can be harvested with successful rate of 100% while avoid the loss of hematopoietic stem cells in UCB [115]; (4) The collection procedure is a non-invasive process without any pain or effect on the mother and fetus; (5) Due to the presence of placental barrier, hUC-MSCs have lower risk of bacterial and viral infections than that MSCs isolated from bone marrow, peripheral blood, fat and etc; (6) With differentiation potential lying between ESCs and ASCs, they do not proliferate unlimitedly or form teratoma while can be successfully induced into mature fat cells, bone cells, cartilage cells, skeletal muscle cells, cardiac cells, endothelial cells, hepatocyte-like cells, islet-like cells, neurons, astrocytes and oligodentrocytes in vitro and in vivo; (7) Preliminary studies have shown that these cells, without expression of MHC II molecules and low expression of MHC I molecules, are likely to be a source of allogeneic cell transplantation.

In view of the above mentioned prominent advantages, the first hUC-MSCs bank complying with Good Manufacturing Practices (GMP) have been established in National Engineering Research Center of Cell Products in Tianjin, China [116]. During the period between May 2006 and December 2008, a total of 5400 umbilical cord were collected and 4428 (82%) were finally stored. The passage-7(P7) were used to manufacture final cell products for animal studies and clinical trials in that as many as approximately 10^{10} cells without signs of senescence or chromosomal abnormalities could be harvested at P7 [116]. Taken together with others [117, 118], these data suggest that UC-MSCs banking will open up interesting new possibilities for cell therapy and regenerative medicine.

However, as MSCs of other origins, the hUC-MSCs are still lack of definitive cell markers for specific isolation and identification which makes a homogenous population of hUC-MSCs is only obtained after several passages [119]. If the suggested novel surface marker, the neural ganglioside GD2, could become generally recognized cell marker for specific isolation and purification of hUC-MSCs in early-passage culture, the isolation procedure will be more efficient [119]. In addition, although several investigations reported the immunosuppressive or immunomodulatory effects of hUC-MSCs [100–102], Cho et al [103] found that hUC-MSCs did elicit an immune response when injected in an inflamed region or injected repeatedly in the same region or stimulated with IFN-γ prior to injection. Therefore, this immune response must be weighted

carefully when considering clinical cellular repair strategies in such circumstances to achieve the desired benefit.

Therefore, improvements in specific isolation, purification and immune characterization of hUC-MSCs may facilitate their clinical application. The promising results in clinical trial in treatment of patients with liver cirrhosis, systemic lupus erythematosus, multiple sclerosis and GVHD after hematopoietic stem cell transplantation for leukemia will further promote the therapeutic application in cell therapy and regenerative medicine [116, 119–122]. Provided with the above-mentioned advantages, hUC-MSCs may become attractive cell sources in tissue engineering and treatment of currently refractory and degenerative diseases.

References

1. Nanaev, A. K., Kohnen, G., Milovanov, A. P., Domogatsky, S. P., & Kaufmann, P. (1997). Stromal differentiation and architecture of the human umbilical cord. *Placenta, 18*(1), 53–64.
2. Eyden, B. P., Ponting, J., Davies, H., Bartley, C., & Torgersen, E. (1994). Defining the myofibroblast: normal tissues, with special reference to the stromal cells of Wharton's jelly in human umbilical cord. *Journal of Submicroscopic Cytology and Pathology, 26*(3), 347–355.
3. Kobayashi, K., Kubota, T., & Aso, T. (1998). Study on myofibroblast differentiation in the stromal cells of Wharton's jelly: expression and localization of alpha-smooth muscle actin. *Early Human Development, 51*(3), 223–233.
4. Mitchell, K. E., Weiss, M. L., Mitchell, B. M., et al. (2003). Matrix cells from Wharton's jelly form neurons and glia. *Stem Cells, 21*(1), 50–60.
5. Romanov, Y. A., Svintsitskaya, V. A., & Smirnov, V. N. (2003). Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. *Stem Cells, 21*(1), 105–110.
6. Can, A., & Karahuseyinoglu, S. (2007). Concise review: human umbilical cord stroma with regard to the source of fetus-derived stem cells. *Stem Cells, 25*(11), 2886–2895.
7. Troyer, D. L., & Weiss, M. L. (2008). Wharton's jelly-derived cells are a primitive stromal cell population. *Stem Cells, 26*(3), 591–599.
8. Hyslop, L. A., Armstrong, L., Stojkovic, M., & Lako, M. (2005). Human embryonic stem cells: biology and clinical implications. *Expert Reviews in Molecular Medicine, 7*(19), 1–21.
9. Geeta, R., Ramnath, R. L., Rao, H. S., & Chandra, V. (2008). One year survival and significant reversal of motor deficits in parkinsonian rats transplanted with hESC derived dopaminergic neurons. *Biochemical and Biophysical Research Communications, 373*(2), 258–264.
10. López-González, R., Knuckles, P., & Velasco, I. (2009). Transient Recovery in a Rat Model of Familial Amyotrophic Lateral Sclerosis after Transplantation of Motor Neurons Derived From Mouse Embryonic Stem Cells. *Cell Transplantation, 18*(10), 1171–1181.
11. Sharp, J., Frame, J., Siegenthaler, M., Nistor, G., & Keirstead, H. S. (2010). Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants improve recovery after cervical spinal cord injury. *Stem Cells, 28*(1), 152–163.
12. Hatami, M., Mehrjardi, N. Z., Kiani, S., et al. (2009). Human embryonic stem cell-derived neural precursor transplants in collagen scaffolds promote recovery in injured rat spinal cord. *Cytotherapy, 11*(5), 618–630.
13. Hicks, A. U., Lappalainen, R. S., Narkilahti, S., et al. (2009). Transplantation of human embryonic stem cell-derived neural precursor cells and enriched environment after cortical stroke in rats: cell survival and functional recovery. *The European Journal of Neuroscience, 29*(3), 562–574.
14. Pal, R. (2009). Embryonic stem (ES) cell-derived cardiomyocytes: a good candidate for cell therapy applications. *Cell Biology International, 33*(3), 325–336.
15. Jiang, J., Au, M., Lu, K., et al. (2007). Generation of insulin-producing islet-like clusters from human embryonic stem cells. *Stem Cells, 25*(8), 1940–1953.
16. Wang, Y., Yates, F., Naveiras, O., Ernst, P., & Daley, G. Q. (2005). Embryonic stem cell-derived hematopoietic stem cells. *Proceedings of the National Academy of Sciences of the United States of America, 102*(52), 19081–6.
17. Ishii, T., Yasuchika, K., Machimoto, T., et al. (2007). Transplantation of embryonic stem cell-derived endodermal cells into mice with induced lethal liver damage. *Stem Cells, 25*(12), 3252–3260.
18. Van Vranken, B.E., Rippon, H.J., Samadikuchaksaraei, A., Trounson, A.O., & Bishop, A.E. (2007). The differentiation of distal lung epithelium from embryonic stem cells. Curr Protoc Stem Cell Biol, Chapter 1, Unit 1G.1.
19. Riekstina, U., Muceniece, R., Cakstina, I., Muiznieks, I., & Ancans, J. (2008). Characterization of human skin-derived mesenchymal stem cell proliferation rate in different growth conditions. *Cytotechnology, 58*(3), 153–162.
20. Gastens, M. H., Goltry, K., Prohaska, W., et al. (2007). Good manufacturing practice-compliant expansion of marrow-derived stem and progenitor cells for cell therapy. *Cell Transplantation, 16*(7), 685–696.
21. Keyser, K. A., Beagles, K. E., & Kiem, H. P. (2007). Comparison of mesenchymal stem cells from different tissues to suppress T-cell activation. *Cell Transplantation, 16*(5), 555–562.
22. Roobrouck, V. D., Ulloa-Montoya, F., & Verfaillie, C. M. (2008). Self-renewal and differentiation capacity of young and aged stem cells. *Experimental Cell Research, 314*(9), 1937–1944.
23. Chang, P. L., Blair, H. C., Zhao, X., et al. (2006). Comparison of fetal and adult marrow stromal cells in osteogenesis with and without glucocorticoids. *Connective Tissue Research, 47*(2), 67–76.
24. Fan, C. G., Tang, F. W., Zhang, Q. J., et al. (2005). Characterization and neural differentiation of fetal lung mesenchymal stem cells. *Cell Transplantation, 14*(5), 311–321.
25. In't Anker, P. S., Noort, W. A., Scherjon, S. A., et al. (2003). Mesenchymal stem cells in human second-trimester bone marrow, liver, lung, and spleen exhibit a similar immunophenotype but a heterogeneous multilineage differentiation potential. *Haematologica, 88*(8), 845–852.
26. Hu, Y., Liao, L., Wang, Q., et al. (2003). Isolation and identification of mesenchymal stem cells from human fetal pancreas. *The Journal of Laboratory and Clinical Medicine, 141*(5), 342–349.
27. Yu, M., Xiao, Z., Shen, L., & Li, L. (2004). Mid-trimester fetal blood-derived adherent cells share characteristics similar to mesenchymal stem cells but full-term umbilical cord blood does not. *British Journal Haematology, 124*(5), 666–675.
28. Lu, F. Z., Fujino, M., Kitazawa, Y., et al. (2005). Characterization and gene transfer in mesenchymal stem cells derived from human umbilical-cord blood. *The Journal of Laboratory and Clinical Medicine, 146*(5), 271–278.
29. Fan, C. G., Zhang, Q. J., & Han, Z. C. (2005). Neural differentiation of mesenchymal stem cells from umbilical cord. *Chinese Journal of Neurosurgery, 21*(7), 388–392.
30. Mareschi, K., Rustichelli, D., Comunanza, V., et al. (2009). Multipotent mesenchymal stem cells from amniotic fluid

originate neural precursors with functional voltage-gated sodium channels. *Cytotherapy, 11*(5), 534–547.
31. In't Anker, P. S., Scherjon, S. A., Kleijburg-van der Keur, C., et al. (2004). Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. *Stem Cells, 22*(7), 1338–1345.
32. Bilic, G., Zeisberger, S. M., Mallik, A. S., Zimmermann, R., & Zisch, A. H. (2008). Comparative characterization of cultured human term amnion epithelial and mesenchymal stromal cells for application in cell therapy. *Cell Transplantation, 17*(8), 955–968.
33. Wang, H. S., Hung, S. C., Peng, S. T., et al. (2004). Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. *Stem Cells, 22*(7), 1330–1337.
34. Karahuseyinoglu, S., Cinar, O., Kilic, E., et al. (2007). Biology of stem cells in human umbilical cord stroma: in situ and in vitro surveys. *Stem Cells, 25*(2), 319–331.
35. Schneider, R. K., Puellen, A., Kramann, R., et al. (2010). The osteogenic differentiation of adult bone marrow and perinatal umbilical mesenchymal stem cells and matrix remodelling in three-dimensional collagen scaffolds. *Biomaterials, 31*(3), 467–480.
36. Wang, L., Tran, I., Seshareddy, K., Weiss, M. L., & Detamore, M. S. (2009). A comparison of human bone marrow-derived mesenchymal stem cells and human umbilical cord-derived mesenchymal stromal cells for cartilage tissue engineering. *Tissue Engineering. Part A, 15*(8), 2259–2266.
37. Karahuseyinoglu, S., Kocaefe, C., Balci, D., Erdemli, E., & Can, A. (2008). Functional structure of adipocytes differentiated from human umbilical cord stroma-derived stem cells. *Stem Cells, 26*(3), 682–691.
38. Woodbury, D., Schwarz, E. J., Prockop, D. J., & Black, I. B. (2000). Adult rat and human bone marrow stromal cells differentiate into neurons. *Journal of Neuroscience Research, 61*(4), 364–370.
39. Fu, Y. S., Shih, Y. T., Cheng, Y. C., & Min, M. Y. (2004). Transformation of human umbilical mesenchymal cells into neurons in vitro. *Journal of Biomedical Science, 11*(5), 652–660.
40. Ma, L., Feng, X. Y., Cui, B. L., et al. (2005). Human umbilical cord Wharton's Jelly-derived mesenchymal stem cells differentiation into nerve-like cells. *Chinese Medical Journal (English), 118*(23), 1987–1993.
41. Ding, D. C., Shyu, W. C., Chiang, M. F., et al. (2007). Enhancement of neuroplasticity through upregulation of beta1-integrin in human umbilical cord-derived stromal cell implanted stroke model. *Neurobiology of Disease, 27*(3), 339–353.
42. Kadam, S. S., Tiwari, S., & Bhonde, R. R. (2009). Simultaneous isolation of vascular endothelial cells and mesenchymal stem cells from the human umbilical cord. *In Vitro Cellular & Developmental Biology Animal, 45*(1–2), 23–27.
43. Weiss, M. L., Medicetty, S., Bledsoe, A. R., et al. (2006). Human umbilical cord matrix stem cells: preliminary characterization and effect of transplantation in a rodent model of Parkinson's disease. *Stem Cells, 24*(3), 781–792.
44. Fu, Y. S., Cheng, Y. C., Lin, M. Y., et al. (2006). Conversion of human umbilical cord mesenchymal stem cells in Wharton's jelly to dopaminergic neurons in vitro: potential therapeutic application for Parkinsonism. *Stem Cells, 24*(1), 115–124.
45. Kadivar, M., Khatami, S., Mortazavi, Y., Shokrgozar, M. A., Taghikhani, M., & Soleimani, M. (2006). In vitro cardiomyogenic potential of human umbilical vein-derived mesenchymal stem cells. *Biochemical and Biophysical Research Communications, 340*(2), 639–647.
46. Koh, S. H., Kim, K. S., Choi, M. R., et al. (2008). Implantation of human umbilical cord-derived mesenchymal stem cells as a neuroprotective therapy for ischemic stroke in rats. *Brain Research, 1229*, 233–248.
47. Lund, R. D., Wang, S., Lu, B., et al. (2007). Cells isolated from umbilical cord tissue rescue photoreceptors and visual functions in a rodent model of retinal disease. *Stem Cells, 25*(3), 602–611.
48. Hess, D. C., & Borlongan, C. V. (2008). Cell-based therapy in ischemic stroke. *Expert Review of Neurotherapeutics, 8*(8), 1193–1201.
49. Skvortsova, V. I., Gubskiy, L. V., Tairova, R. T., et al. (2008). Use of bone marrow mesenchymal (stromal) stem cells in experimental ischemic stroke in rats. *Bulletin of Experimental Biology and Medicine, 145*(1), 122–128.
50. Liao, W., Xie, J., Zhong, J., et al. (2009). Therapeutic effect of human umbilical cord multipotent mesenchymal stromal cells in a rat model of stroke. *Transplantation, 87*(3), 350–359.
51. Liao, W., Zhong, J., Yu, J., et al. (2009). Therapeutic benefit of human umbilical cord derived mesenchymal stromal cells in intracerebral hemorrhage rat: implications of anti-inflammation and angiogenesis. *Cellular Physiology and Biochemistry, 24*(3–4), 307–316.
52. Deshpande, D. M., Kim, Y. S., Martinez, T., et al. (2006). Recovery from paralysis in adult rats using embryonic stem cells. *Annals of Neurology, 60*(1), 32–44.
53. Lim, J. H., Byeon, Y. E., Ryu, H. H., et al. (2007). Transplantation of canine umbilical cord blood-derived mesenchymal stem cells in experimentally induced spinal cord injured dogs. *Journal of Veterinary Science, 8*(3), 275–282.
54. Zhao, Z. M., Li, H. J., Liu, H. Y., et al. (2004). Intraspinal transplantation of CD34+ human umbilical cord blood cells after spinal cord hemisection injury improves functional recovery in adult rats. *Cell Transplantation, 13*(2), 113–122.
55. Coutts, M., & Keirstead, H. S. (2008). Stem cells for the treatment of spinal cord injury. *Experimental Neurology, 209*(2), 368–377.
56. Yang, C. C., Shih, Y. H., Ko, M. H., Hsu, S. Y., Cheng, H., & Fu, Y. S. (2008). Transplantation of human umbilical mesenchymal stem cells from Wharton's jelly after complete transection of the rat spinal cord. *PLoS ONE, 3*(10), e3336.
57. Zhang, L., Zhang, H. T., Hong, S. Q., Ma, X., Jiang, X. D., & Xu, R. X. (2009). Cografted Wharton's jelly cells-derived neurospheres and BDNF promote functional recovery after rat spinal cord transection. *Neurochemical Research, 34*(11), 2030–2039.
58. Yasuhara, T., & Date, I. (2007). Intracerebral transplantation of genetically engineered cells for Parkinson's disease: toward clinical application. *Cell Transplantation, 16*(2), 125–132.
59. Zhang, S., Liu, X. Z., Liu, Z. L., et al. (2009). Stem cells modified by brain-derived neurotrophic factor to promote stem cells differentiation into neurons and enhance neuromotor function after brain injury. *Chinese Journal of Traumatology, 12*(4), 195–199.
60. Zimmet, P., Alberti, K. G., & Shaw, J. (2001). Global and societal implications of the diabetes epidemic. *Nature, 414*, 782–787.
61. Bretzel, R. G., Browatzki, C. C., Schultz, A., et al. (1993). Clinical islet transplantation in diabetes mellitus-report of the Islet Transplant Registry and the Giessen Center experience. *Diabet Stoffwechsel, 2*, 378–390.
62. Shapiro, A. M., Lakey, J. R., Ryan, E. A., et al. (2000). Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *The New England Journal of Medicine, 343*, 230–238.
63. Lumelsky, N., Blondel, O., Laeng, P., et al. (2001). Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. *Science, 292*, 1389–1394.
64. Ramiya, V. K., Maraist, M., Arfors, K. E., et al. (2000). Reversal of insulin-dependent diabetes using islets generated in vitro from pancreatic stem cells. *Natural Medicines, 6*, 278–282.

65. Yang, L., Li, S., Hatch, H., et al. (2002). In vitro trans-differentiation of adult hepatic stem cells into pancreatic endocrine hormoneproducing cells. *Proceedings of the National Academy of Sciences of the United States of America, 99*, 8078–8083.
66. Hori, Y., Gu, X., Xie, X., et al. (2005). Differentiation of insulin-producing cells from human neural progenitor cells. *PLoS Medicine, 2*(4), e103.
67. Oh, S. H., Muzzonigro, T. M., Bae, S. H., et al. (2004). Adult bone marrow-derived cells trans-differentiating into insulin-producing cells for the treatment of type I diabetes. *Laboratory Investigation, 84*, 607–617.
68. Ende, N., Chen, R., & Reddi, A. S. (2004). Effect of human umbilical cord blood cells on glycemia and insulitis in type 1 diabetic mice. *Biochemical and Biophysical Research Communications, 325*, 665–669.
69. Chao, K. C., Chao, K. F., Fu, Y. S., & Liu, S. H. (2008). Islet-like clusters derived from mesenchymal stem cells in Wharton's Jelly of the human umbilical cord for transplantation to control type 1 diabetes. *PLoS ONE, 3*(1), e1451.
70. Lorenzini, S., Gitto, S., Grandini, E., Andreone, P., & Bernardi, M. (2008). Stem cells for end stage liver disease: how far have we got? *World Journal of Gastroenterology, 14*(29), 4593–4599.
71. Campard, D., Lysy, P. A., Najimi, M., & Sokal, E. M. (2008). Native umbilical cord matrix stem cells express hepatic markers and differentiate into hepatocyte-like cells. *Gastroenterology, 134*(3), 833–848.
72. Zhang, Y. N., Lie, P. C., & Wei, X. (2009). Differentiation of mesenchymal stromal cells derived from umbilical cord Wharton's jelly into hepatocyte-like cells. *Cytotherapy, 11*(5), 548–558.
73. Zhao, Q., Ren, H., Li, X., et al. (2009). Differentiation of human umbilical cord mesenchymal stromal cells into low immunogenic hepatocyte-like cells. *Cytotherapy, 11*(4), 414–426.
74. Tsai, P. C., Fu, T. W., Chen, Y. M., et al. (2009). The therapeutic potential of human umbilical mesenchymal stem cells from Wharton's jelly in the treatment of rat liver fibrosis. *Liver Transplant, 15*(5), 484–495.
75. Yan, Y., Xu, W., Qian, H., et al. (2009). Mesenchymal stem cells from human umbilical cords ameliorate mouse hepatic injury in vivo. *Liver International, 29*(3), 356–365.
76. Kadner, A., Hoerstrup, S. P., Tracy, J., et al. (2002). Human umbilical cord cells: a new cell source for cardiovascular tissue engineering. *The Annals of Thoracic Surgery, 74*(4), S1422–1428.
77. Kadner, A., Zund, G., Maurus, C., et al. (2004). Human umbilical cord cells for cardiovascular tissue engineering: a comparative study. *European Journal of Cardiothoracic Surgery, 25*(4), 635–641.
78. Hoerstrup, S. P., Kadner, A., Breymann, C., et al. (2002). Living, autologous pulmonary artery conduits tissue engineered from human umbilical cord cells. *The Annals of Thoracic Surgery, 74*(1), 46–52.
79. Schmidt, D., Mol, A., Neuenschwander, S., et al. (2005). Living patches engineered from human umbilical cord derived fibroblasts and endothelial progenitor cells. *European Journal of Cardiothoracic Surgery, 27*(5), 795–800.
80. Sodian, R., Lueders, C., Kraemer, L., et al. (2006). Tissue engineering of autologous human heart valves using cryopreserved vascular umbilical cord cells. *The Annals of Thoracic Surgery, 81*(6), 2207–2216.
81. Breymann, C., Schmidt, D., & Hoerstrup, S. P. (2006). Umbilical cord cells as a source of cardiovascular tissue engineering. *Stem Cell Reviews, 2*(2), 87–92.
82. Pereira, W. C., Khushnooma, I., Madkaikar, M., & Ghosh, K. (2008). Reproducible methodology for the isolation of mesenchymal stem cells from human umbilical cord and its potential for cardiomyocyte generation. *Journal of Tissue Engineering and Regenerative Medicine, 2*(7), 394–399.
83. Martin-Rendon, E., Sweeney, D., Lu, F., Girdlestone, J., Navarrete, C., & Watt, S. M. (2008). 5-Azacytidine-treated human mesenchymal stem/progenitor cells derived from umbilical cord, cord blood and bone marrow do not generate cardiomyocytes in vitro at high frequencies. *Vox Sanguinis, 95*(2), 137–148.
84. Wu, K. H., Zhou, B., Lu, S. H., et al. (2007). In vitro and in vivo differentiation of human umbilical cord derived stem cells into endothelial cells. *Journal of Cellular Biochemistry, 100*(3), 608–616.
85. Conconi, M. T., Burra, P., Di Liddo, R., et al. (2006). CD105(+) cells from Wharton's jelly show in vitro and in vivo myogenic differentiative potential. *International Journal of Molecular Medicine, 18*(6), 1089–1096.
86. Chen, M. Y., Lie, P. C., Li, Z. L., & Wei, X. (2009). Endothelial differentiation of Wharton's jelly-derived mesenchymal stem cells in comparison with bone marrow-derived mesenchymal stem cells. *Experimental Hematology, 37*(5), 629–640.
87. Wang, S. H., Lin, S. J., Chen, Y. H., et al. (2009). Late outgrowth endothelial cells derived from Wharton jelly in human umbilical cord reduce neointimal formation after vascular injury: involvement of pigment epithelium-derived factor. *Arteriosclerosis, Thrombosis, and Vascular Biology, 29*(6), 816–822.
88. Li, N., Feugier, P., Serrurrier, B., et al. (2007). Human mesenchymal stem cells improve ex vivo expansion of adult human CD34+ peripheral blood progenitor cells and decrease their allostimulatory capacity. *Experimental Hematology, 35*(3), 507–515.
89. Kim, D. W., Chung, Y. J., Kim, T. G., Kim, Y. L., & Oh, I. H. (2004). Cotransplantation of third-party mesenchymal stromal cells can alleviate single-donor predominance and increase engraftment from double cord transplantation. *Blood, 103*(5), 1941–1948.
90. Lu, L. L., Liu, Y. J., Yang, S. G., et al. (2006). Isolation and characterization of human umbilical cord mesenchymal stem cells with hematopoiesis-supportive function and other potentials. *Haematologica, 91*(8), 1017–1026.
91. Bakhshi, T., Zabriskie, R. C., Bodie, S., et al. (2008). Mesenchymal stem cells from the Wharton's jelly of umbilical cord segments provide stromal support for the maintenance of cord blood hematopoietic stem cells during long-term ex vivo culture. *Transfusion, 48*(12), 2638–2644.
92. Friedman, R., Betancur, M., Boissel, L., Tuncer, H., Cetrulo, C., & Klingemann, H. (2007). Umbilical cord mesenchymal stem cells: adjuvants for human cell transplantation. *Biology of Blood and Marrow Transplantation, 13*(12), 1477–1486.
93. Hao, M., Meng, H. X., Li, G., et al. (2009). Study of influence of umbilical cord mesenchymal stem cells on $CD34^{+}$ cells in vivo homing in NOD/SCID. *Zhonghua Xue Ye Xue Za Zhi, 30*(2), 103–106.
94. Liu, M., Yang, S. G., Liu, P. X., et al. (2009). Comparative study of in vitro hematopoietic supportive capability of human mesenchymal stem cells derived from bone marrow and umbilical cord. *Zhongguo Shi Yan Xue Ye Xue Za Zhi, 17*(5), 1294–1300.
95. Chao, K. C., Chao, K. F., Chen, C. F., & Liu, S. H. (2008). A novel human stem cell coculture system that maintains the survival and function of culture islet-like cell clusters. *Cell Transplantation, 17*(6), 657–664.
96. Boissel, L., Tuncer, H. H., Betancur, M., Wolfberg, A., & Klingemann, H. (2008). Umbilical cord mesenchymal stem cells increase expansion of cord blood natural killer cells. *Biology of Blood and Marrow Transplantation, 14*(9), 1031–1038.

97. Skottman, H., & Hovatta, O. (2006). Culture conditions for human embryonic stem cells. *Reproduction, 132*(5), 691–698.
98. Hiroyama, T., Sudo, K., Aoki, N., et al. (2008). Human umbilical cord-derived cells can often serve as feeder cells to maintain primate embryonic stem cells in a state capable of producing hematopoietic cells. *Cell Biology International, 32*(1), 1–7.
99. Salgado, A. J., Fraga, J. S., Mesquita, A. R., Neves, N. M., Reis, R. L., & Sousa, N. (2009). Role of Human Umbilical Cord Mesenchymal Progenitors Conditioned Media in Neuronal/Glial Cell Densities. Viability and Proliferation. *Stem Cells and Development*. doi:10.1089/scd.2009.0279.
100. Nauta, A. J., & Fibbe, W. E. (2007). Immunomodulatory properties of mesenchymal stromal cells. *Blood, 110*(10), 3499–3506.
101. Weiss, M. L., Anderson, C., Medicetty, S., et al. (2008). Immune properties of human umbilical cord Wharton's jelly-derived cells. *Stem Cells, 26*(11), 2865–2874.
102. Yoo, K. H., Jang, I. K., Lee, M. W., et al. (2009). Comparison of immunomodulatory properties of mesenchymal stem cells derived from adult human tissues. *Cellular Immunology, 259* (2), 150–156.
103. Cho, P. S., Messina, D. J., Hirsh, E. L., et al. (2008). Immunogenicity of umbilical cord tissue derived cells. *Blood, 111*(1), 430–438.
104. Kumar, S., Chanda, D., & Ponnazhagan, S. (2008). Therapeutic potential of genetically modified mesenchymal stem cells. *Gene Therapy, 15*(10), 711–715.
105. Fritz, V., & Jorgensen, C. (2008). Mesenchymal stem cells: an emerging tool for cancer targeting and therapy. *Current Stem Cell Research & Therapy, 3*(1), 32–42.
106. Baksh, D., Yao, R., & Tuan, R. S. (2007). Comparison of proliferative and multilineage differentiation potential of human mesenchymal stem cells derived from umbilical cord and bone marrow. *Stem Cells, 25*(6), 1384–1392.
107. Rachakatla, R. S., Pyle, M. M., Ayuzawa, R., et al. (2008). Combination treatment of human umbilical cord matrix stem cell-based interferon-beta gene therapy and 5-fluorouracil significantly reduces growth of metastatic human breast cancer in SCID mouse lungs. *Cancer Investigation, 26*(7), 662–670.
108. Rachakatla, R. S., Marini, F., Weiss, M. L., Tamura, M., & Troyer, D. (2007). Development of human umbilical cord matrix stem cell-based gene therapy for experimental lung tumors. *Cancer Gene Therapy, 14*(10), 828–835.
109. Chen, X. L., Dong, C. L., Feng, X. M., et al. (2009). Expression of human factor IX in retrovirus-transfected human umbilical cord tissue derived mesenchymal stem cells. *Zhongguo Shi Yan Xue Ye Xue Za Zhi, 17*(1), 184–187.
110. Lee, D. H., Ahn, Y., Kim, S. U., et al. (2009). Targeting rat brainstem glioma using human neural stem cells and human mesenchymal stem cells. *Clinical Cancer Research, 15*(15), 4925–4934.
111. Stolzing, A., Jones, E., McGonagle, D., & Scutt, A. (2008). Age-related changes in human bone marrow-derived mesenchymal stem cells: consequences for cell therapies. *Mechanisms of Ageing and Development, 129*(3), 163–173.
112. Zhou, S., Greenberger, J. S., Epperly, M. W., et al. (2008). Age-related intrinsic changes in human bone-marrow-derived mesenchymal stem cells and their differentiation to osteoblasts. *Aging Cell, 7*(3), 335–343.
113. Rao, M. S., & Mattson, M. P. (2001). Stem cells and aging: expanding the possibilities. *Mechanisms of Ageing and Development, 122*(7), 713–734.
114. Petsa, A., Gargani, S., Felesakis, A., Grigoriadis, N., & Grigoriadis, I. (2009). Effectiveness of protocol for the isolation of Wharton's Jelly stem cells in large-scale applications. *In Vitro Cellular & Developmental Biology. Animal, 45*(10), 573–576.
115. Shetty, P., Cooper, K., & Viswanathan, C. (2010). Comparison of proliferative and multilineage differentiation potentials of cord matrix, cord blood, and bone marrow mesenchymal stem cells. *Asian Journal Transfusion Science, 4*(1), 14–24.
116. Han, Z. C. (2009). Umblical cord mesenchymal stem cells (UC-MSC: biology, banking and clinical applications). *Bulletin de l'Académie Nationale de Médecine, 193*(3), 545–547. discussion 547.
117. Secco, M., Zucconi, E., Vieira, N. M., et al. (2008). Mesenchymal stem cells from umbilical cord: do not discard the cord! *Neuromuscular Disorders, 18*(1), 17–18.
118. Secco, M., Zucconi, E., Vieira, N. M., et al. (2008). Multipotent stem cells from umbilical cord: cord is richer than blood! *Stem Cells, 26*(1), 146–150.
119. Xu, J., Liao, W., Gu, D., et al. (2009). Neural ganglioside GD2 identifies a subpopulation of mesenchymal stem cells in umbilical cord. *Cellular Physiology and Biochemistry, 23*(4–6), 415–424.
120. Liang, J., Gu, F., Wang, H., et al. (2010). Mesenchymal stem cell transplantation for diffuse alveolar hemorrhage in SLE. *Nature Reviews Rheumatology*. doi:10.1038/nrrheum.2010.80.
121. Sun, L., Wang, D., Liang, J., et al. (2010). Umbilical cord mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus. *Arthritis and Rheumatism*. doi:10.1177/1352458509104590.
122. Liang, J., Zhang, H., Hua, B., et al. (2009). Allogeneic mesenchymal stem cells transplantation in treatment of multiple sclerosis. *Multiple Sclerosis, 15*(5), 644–646.