Front. Med. 2015, 9(1): 20–29
DOI 10.1007/s11684-014-0371-x

REVIEW

Implantation of human umbilical cord mesenchymal stem cells for ischemic stroke: perspectives and challenges

Yingchen Li[1], Guoheng Hu (✉)[2], Qilai Cheng[1]

[1]*Post-Graduate School, Hunan University of Traditional Chinese Medicine, Changsha 410208, China;* [2]*Department of Neurology, The First Affiliated Hospital of Hunan University of Traditional Chinese Medicine, Changsha 410007, China*

Abstract **Ischemic stroke is a focal cerebral insult that often leads to many adverse neurological complications severely affecting the quality of life. The prevalence of stroke is increasing throughout the world, while the efficacy of current pharmacological therapies remains unclear. As a neuroregenerative therapy, the implantation of human umbilical cord mesenchymal stem cells (hUC-MSCs) has shown great possibility to restore function after stroke. This review article provides an update role of hUC-MSCs implantation in the treatment of ischemic stroke. With the unique "immunosuppressive and immunoprivilege" property, hUC-MSCs are advised to be an important candidate for allogeneic cell treatment. Nevertheless, most of the treatments are still at primary stage and not clinically feasible at the current time. Several uncertain problems, such as culture conditions, allograft rejection, and potential tumorigenicity, are the choke points in this cellular therapy. More preclinical researches and clinical studies are needed before hUC-MSCs implantation can be used as a routinely applied clinical therapy.**

Keywords human umbilical cord; mesenchymal stem cells; ischemic stroke; cellular therapy; transplantation

Introduction

Ischemic stroke is one of the leading causes of motor, sensory, cognitive dysfunctions and death all over the world. Unfortunately, except the short time window required thrombolytic therapy, to date no effective treatment is proved to promote neuronal function recovery.

In the latest decades, stem cell-based therapies have become a potential approach to restore function in stroke [1]. Human umbilical cord mesenchymal stem cell (hUC-MSC) is a unique, accessible, and non-controversial source of stem cell which can enhance neurogenesis and angiogenesis in animal models of ischemic stroke [2]. In this review, we collected data on mechanisms and strategies of MSCs-based treatment, and evaluated the potentials and challenges of hUC-MSCs transplantation for ischemic stroke.

Received February 24, 2014; accepted August 12, 2014
Correspondence: yingchen4@163.com

Sources of stem cells

Various types of stem cells have been isolated from a variety of tissue including preimplantation embryos, fetuses, birth-associated tissue and adult organs. Based on biochemical and genomic markers, these cells can be broadly classified into embryonic stem cells (ESCs), mesenchymal stem cells (MSCs), and hematopoietic stem cells (HPSCs).

ESCs are pluripotent stem cells which theoretically can differentiate into all cell types of human body. However, ESCs suffer from a series of constraints including ethical concerns, limited availability, potential for teratoma formation and immune rejection [3]. Induced pluripotent stem cell (iPSC) is an alternative to ESCs, and has been generated from many cell types including cells from umbilical cord (UC) [4]. Although the use of patient-specific iPSCs will overcome the immune rejection obstacle, a great deal of work is needed before these cells can be used as a routinely applied therapy. The risk of tumorigenesis is particular important when using iPSCs, which are characterized by the ability to form teratomas in animal models [5].

HPSCs have limited plasticity since they can only differentiate into blood and blood-related lineages. In

addition, there are only a small number of HPSCs in bone marrow and umbilical cord (UC), the cells require *ex vivo* expansion for clinical application [6].

Fetal MSCs are controversial as they are derived from human abortuses. Since the group of Pittenger isolated the multipotent MSCs from bone marrow first, the bone marrow has become the primary source to obtain MSCs [6]. Bone marrow mesenchymal stem cells (BM-MSCs) are the most extensively investigated MSCs and are considered the "gold standard" cell source in regenerative medicine. However, there are some limitations in the applications of BM-MSCs, including the small quantity of MSCs in marrow, the significant decrease of proliferation and differentiation potential with age. Moreover, the invasive harvesting procedure may lead to complications and morbidity [7].

Extra-embryonic MSCs harvested from UC represent a special stem cell type that combines some pluripotent properties of adult MSCs. With the close ontogenetic relationship to ESCs, hUC-MSCs have an immunoprivilege characteristic and proliferate faster than adult MSCs [8]. In addition, these cells can be isolated and used without any ethical problems, since extra-embryonic tissue is normally discarded after birth.

Isolation and culture of hUC-MSCs

hUC-MSCs can be derived from perivascular tissue and Wharton's jelly in UC [9]. Wharton's jelly means the mucoid connective substance which surrounds and supports the blood vessels in the cord. In 2003, Romanov *et al.* isolated the cells expressing MSCs markers from human umbilical cord first. However, the isolation and culture of hUC-MSCs are still controversial now. There are mainly two techniques: explant culture and enzymatic digestion, while the first way is more popular. In explant culture, which is also called "plate and wait," the cord segments are simply plated in the medium to wait for the MSCs to migrate out [10]. Enzymatic digestion method means digesting Wharton's jelly in an enzyme solution to release and obtain MSCs. The former technique is simple, effective, inexpensive and less labor-intense, but it is also time-consuming. The latter is more efficient when compared with explant culture, but overdigestion of tissue may result in diminished cellular viability, and altered cellular function [11]. There is a modified enzymatic digestion method which is more productive and economical: in the first step, the whole cord was digested to migrate vascular endothelial cells out, and then the rest of the cord was digested again to isolate the MSCs [12]. Besides, Dong *et al.* reported that the combination of collagenase type II, collagenase type IV, hyaluronidase, trypsin and DNAase could significantly improve the yield, viability and proliferation ability of MSCs, and the time of isolation could be shortened [13]. Liu *et al.* suggested that the implantation efficiency of hUC-MSCs could be increased when the cells were incubated in human umbilical cord serum, and the possible mechanism may be the reduction of senescent process through upregulating the heat shock protein 27 (Hsp27) [14].

Biological characteristics of hUC-MSCs

Surface markers

According to the statement by Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy in 2006, the characteristics of human MSCs should be defined in three aspects: *in vitro* growth pattern, the expression of specific surface antigens (positive for CD105, CD73 and CD90, and negative for CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR surface molecules), and the multipotent differentiation potential [15]. In the reported studies about hUC-MSCs, different reproducible expressions of cell surface epitope markers have been demonstrated by flow cytometric analysis. There are mainly four categories of surface markers which are shown in Table 1 [16–21]. Nevertheless, the defining immunological markers of hUC-MSCs are still inconsistent among investigators. A standardized method should be identified to assess the implantation and immunomodulatory function of hUC-MSCs quickly and efficiently [22].

Immune properties

MSCs have two peculiar and outstanding immunological characteristics: immunosuppression and immunoprivilege [23,24]. They can secrete some soluble factors (such as IDO, sHLA-G5) to suppress the function of lymphocytes and natural killer (NK) cells. By inhibiting the dendritic cells, they can induce regulatory T cells in central nervous

Table 1 Categories of surface markers expressed by hUC-MSCs

Categories	Positive	Negative
Adhesion molecule	CD166, CD54, CD102, CD58, CD56, CD44, CD106	CD62P, CD31
Growth factors and cytokine receptors	CD121, CD123, CD126, CD127, CDw119, CD120a, CD140a	CD25
Integrin families	CD49a, CD49b, CD29, CD104	CD11a and CD11b
Other molecules	SH2, SH3, SH4, Thy1(CD90), CD73, CD105, CD146	CD34, CD45, CD80, CD86

system inflammatory diseases [25]. Furthermore, MSCs are capable of changing the secretion of dendritic cells, T cells, and NK cells, resulting in the feature of anti-inflammatory or tolerant phenotype [26]. There is *in vitro* evidence that MSCs can prevent the antigen-presenting cells from maturing and migrating, and they can change the expression of receptors that are important for antigen capture and processing [16,27]. These studies have presented an attractive cellular therapy for ischemic stroke, and hUC-MSCs have shown great potential since they are easily and non-invasively available without ethical constraints [28,29].

Except for the ability to modulate allogeneic immune system, hUC-MSCs do not appear to form teratomas, since they can express low levels of pluripotent embryonic stem cell markers (such as POUF1, NANOG, SOX2 and LIN28) [30–33]. Meanwhile, hUC-MSCs could upregulate several cytokines (such as IL12A) which are associated with the induction of apoptosis, bringing on their anti-cancer feature [34]. Compared with other kinds of MSCs, hUC-MSCs are found to show increased expression of genes associated with cell chemotaxis and apoptosis. In addition, the immunomodulatory effects of hUC-MSCs did not change in response to neural induction [16]. This activity may play a key role in the rehabilitation of nervous system injuries.

Experimental studies about hUC-MSCs implantation in ischemic stroke

A number of researchers have reported the benefits of hUC-MSCs implantation in cerebral ischemia experiments. The animal model commonly applied is the transient rodent middle cerebral artery occlusion (MCAO) model. In the surgery, the right common carotid artery, external carotid artery and internal carotid artery were exposed. A nylon suture, with its tip rounded by heating near a flame, was advanced from the external carotid artery into the lumen of the internal carotid artery until the origin of the middle cerebral artery was blocked. Several hours later, animals were reanesthetized with isoflurane and blood reperfusion was achieved by withdrawal of the suture [35]. The benefits of neuroprotection were afforded by intravenous administration or intracerebral transplantation of hUC-MSCs, and the time lapse between injury and transplantation was different in the studies.

Reported by Liao *et al.*, the rats were subjected to 2-h MCAO and received intracerebral transplantation of hUC-MSCs 24 h after surgery [36]. The transplanted cells could survive for at least 5 weeks in the brain, and this treatment could significantly reduce the injury volume of brain and neurological functional deficits. In addition, the therapy could substantially increase the vascular density and vascular endothelial growth factor (VEGF) expression in the cerebral hemisphere of infarction.

Similarly, the effectiveness of intravenous administration of hUC-MSCs was tested in rodent MCAO models in a time-of-administration study (time after stroke: day 1, day 7, day 30, and day 90) [1]. Marked functional recovery was observed when treatment was initiated at day 1, 7 or 30 but not at day 90 after stroke. The treatment could also enhance synaptogenesis, vessel density and the proliferation of SVZ cells, and apoptosis in the ischemic area was reduced. Lin *et al.* [37] found that rats receiving hUC-MSCs by intracerebral administration showed significant improvements in motor function, metabolic activity of cortical neurons and revascularization in the infarct cortex. The implanted cells survived in brain for at least 36 days and released neuroprotective and growth-associated cytokines, including brain-derived neurotrophic factor, platelet-derived growth factor-AA, basic fibroblast growth factor, angiopoietin-2, CXCL-16, neutrophil-activating protein-2, and VEGF receptor-3.

Potential therapeutic mechanisms

Replacement of damaged nervous cells

Prior study has indicated that neuronal replacement might be the possible mechanism of cell therapy in Parkinson's disease [38]. There is evidence to show that the transplanted ESCs-derived neural stem cells could survive and differentiate into functional neurons, contributing to behavioral improvements and function restoration in experimental stroke models [39]. However, it may not be realistic that MSCs improve the neurological function of patients through a neuronal replacement mechanism [40]. Intracerebral transplanted MSCs derived from human umbilical cord blood could migrate to hippocampus and alleviate the ischemic brain injury in rats, but only the differentiation of MSCs to astrocytes was observed by histochemical study [41].

Proved by experiments, hUC-MSCs can differentiate into neural progenitors or neuron-like cells with the expression of neuron-specific markers such as nestin, Musashi-1, glial fibrillary acidic protein (GFAP), β-tubulin III, neuron specific protein TH, and neuron specific enolase (NSE) [42,43]. In an *in vivo* study [44], intracerebral implantation of hUC-MSCs improved neurobehavioral function and reduced the infarct volume in rats with ischemic stroke. Three weeks after implantation, most of the implanted hUC-MSCs were present in the damaged hemisphere, and some of these cells expressed detectable levels of neuron-specific markers. However, functional active neuronal signal pathways were not detected by electrophysiological examination, indicating that the benefits of cell transplantation might be related to the

neuroprotective effects rather than the formation of a new network between host neurons and the implanted cells.

It is demonstrated that hUC-MSCs could induce the immune response of host body under inflammatory conditions elicited by stroke, and the transplanted cells could be scavenged by the host immune cells. Weiss *et al*. [45] showed that hUC-MSCs were discovered 2 days after transplantation while no positive-staining hUC-MSCs were found 6 weeks later. The result indicated that the graft cells were cleared by the host immune system, the low-density graft was not able to support itself and the cells could not survive for a long time. Zhang *et al.* [1] reported that few hUC-MSCs were detected 2 months after intravenous transplantation in rat MCAO models, and they suggested that the implanted cells were possibly be eliminated by host immune system. Therefore, although hUC-MSCs have potentials to differentiate into neurons, neural differentiation and integration are unlikely to be the main mechanism of therapeutical function in cell therapy for stroke.

Promotion of endogenous neural cells

In the adult's brain, the subventricular zone (SVZ) of lateral ventricle contains self-renewing multi-potent neural progenitors that are able to generate neurons, astrocytes and oligodentrocytes. Under normal conditions, glial fibrillary acidic protein (GFAP)-expressing cells in the SVZ include a neurogenic cell population that gives rise to olfactory bulb neurons only. Prior researches indicated that stroke increased neurogenesis in the SVZ, and newly generated neurons started to migrate toward ischemic boundary regions soon after stroke attacking. The generation of new neurons involves proliferation of progenitors, subsequent withdrawal from the cell cycle and differentiation [46]. After stroke, neural progenitor cells can proliferate and generate neuroblasts, migrate to the damaged area, become integrated, and differentiate to mature and functional neurons [45,47,48]. On the other hand, Jin *et al.* reported that suppressed SVZ neurogenesis led to worse functional outcome after experimental stroke in mice [49]. Raber *et al.* suggested irradiation in gerbils attenuated neurogenesis and exacerbated deficits of behavioral performance after cerebral global ischemia [50]. SVZ is the main source of new generated neurons, and the recovery after stroke may possibly be induced by SVZ progenitor proliferation and neurogenesis [51].

Besides, it is also reported that large amount of new born neurons eventually underwent cell death in stroke, while the transplanted MSCs could enhance proliferation of endogenous neurogenesis by suppressing apoptosis of newly generated cells [52]. Studies indicated that therapeutic effects of MSCs were at least partly ascribed to enhancing endogenous neurogenesis and protecting newborn cells from deleterious environment. MSCs were reported to promote the proliferation of neural stem cells or progenitor cells in SVZ, increase the expression of genes related to neural differentiation and protect the differentiated neurons [52,53]. In the animal models of MCAO, intravenous transplantation of hUC-MSCs enhanced generation and neuronal differentiation of intrinsic cells in the brain, and made improvements on functional outcomes [1]. Taken together, stroke-induced neurogenesis and neuronal differentiation play important roles in brain repair and recovery, and the effects could be enhanced by hUC-MSCs transplantation.

Secretion of neurotrophic factors

The expression of neuroprotective and growth-associated factors were identified in MSCs, which contributed to the protective effect in tissue repair [54]. The implanted MSCs could improve the survival of host cells and promote regeneration of endogenous cells by factors excretion [55]. In prior researches, human MSCs were known to secrete biologically active cytokines or factors including brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor, glial cell line-derived neurotrophic factor (GDNF), nerve growth factor (NGF), colony-stimulating fator (CSF), stem cell factor (SCF), basic fibroblast growth factor (bFGF), platelet-derived growth factor-AA (PDGF-AA), angiopoietin-2, neutrophil-activating protein-2 (NAP2), and vascular endothelial growth factor receptor-3 (VEGFR-3) [56–58]. Furthermore, it was shown that the secretion of trophic factors by MSCs was enhanced under post-ischemic conditions [59].

Transplanted through the basilar artery to canine ischemic stroke models, MSCs were revealed to successfully integrate into host brains, reduce infarction volume and ameliorate neurobehavioral function, which are closely related to factors releasing [60]. In the *in vivo* studies, hUC-MSCs implantation were able to improve the internal environment of the injured brain, enhance the survival of engrafted cells, and induce neurobehavioral improvement through the paracrine mechanism [35,37]. In addition, the antiapoptotic signaling pathway was found to be activated by neurotrophic factors (such as BDNF), which induced reduction of cell death in brain tissue of stroke models [61]. Intravenous hUC-MSCs administration could also enhance angiogenesis, which was thought to be linked to the paracrine neurotrophic action of the implanted cells [62]. It could be concluded that neurotrophic factors released by MSCs are able to induce endogenous neuronal proliferation and differentiation, promote neovascularization and survival, decrease apoptosis, and bring on earlier recovery of neurological functions after brain injury [62,63].

Induction of vascularization and angiogenesis

Suggested by prevenient researchers, vasculature plays a significant role in striatal neurogenesis after stroke. When stroke attacks, the vascular endothelial cells in brain start to proliferate, and more oxygen and nutrient were supplied to the ischemic tissue. During the next several months, neuroblasts gradually migrate toward the injured brain region with vascular remodeling and vessel density increasing. Therefore, the generation of new blood vessels is quite important for the survival, migration and differentiation of the closely located neuroblasts [64]. As a physiological repair process that naturally occurs in ischemic stroke, changes in vascularization can be promoted by MSCs transplantation. Existing evidence suggested MSCs could be induced to acquire the angiogenic and vasculogenic properties by producing appropriate cytokine milieu [65]. Through the promotion of vascular growth, MSCs could improve cerebral blood flow, and finally ameliorate the ischemic tissue damage [66].

On the other hand, the endothelial potential of hUC-MSCs has been confirmed in experiments. These cells were found to differentiate into vascular endothelial cells with additionally increasing expression of VEGF [67]. Moreover, Zhang *et al*. [1] found that hUC-MSCs were able to modulate the vascular system by increasing density of von Willebrand factor (vWF)-immunoreactive vessels. These studies seems to point toward the theory that administered hUC-MSCs induce angiogenesis and enhance brain blood supplying to reduce stroke-induced deficits, which is likely due to secreting paracrine factors.

Reduction of apoptosis

Neuron apoptosis have been detected in normal adult animal striatum by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling assay (TUNEL assay). Observations suggested that neuronal programmed elimination was a common feature in the adult mammalian brain, in which cells from neurogenic regions were largely eliminated through a self-renewal mechanism [68]. Previous study has demonstrated that stroke-induced cell apoptosis peaked at 1 to 2 days after stroke onset and last for at least 4 weeks, which significantly contributed to enormous death of newly generated cells (approximately 80% of the total) in ischemic brain injury [69].

Transplanted into rats with transient middle cerebral artery occlusion, MSCs could contribute to motor function improvement by reducing neuronal damage via inhibiting neuronal apoptosis [70]. Scheibe *et al*. reported that neuron apoptosis could be prevented and neuronal damage could be reduced by MSCs transplantation in oxygen-glucose deprivation (OGD) model of neurons [71]. hUC-MSCs treatment could promote endogenous neurorestoration and decrease apoptotic cell death till 2 months after stroke [1]. Additionally, it is proved that the capacity of MSCs to prevent apoptosis mainly depended on inactivating some proteolytic enzymes, such as metalloproteinases (MMPs) [72]. It is reasonable to speculate that, through an anti-apoptosis mechanism, MSCs can improve the survival of newly generated cells and repair the injured brain tissue after cerebral ischemia [54].

Prevention of inflammatory effect

Inflammation plays a critical part in ischemic stroke. When the stroke occurs, the brain will show a rapid and prolonged response of inflammatory process such as the activation of resident cells (mainly microglia), production of inflammatory factors and stimulation of various types of inflammatory cells (neutrophils, T cells, monocytes, and macrophages) [73]. All of these cellular events collaboratively contribute to cerebral injury eventually. Therefore, the inhibition of splenic function may be another mechanism of MSCs therapy for stroke [74].

It is reported that spleen adversely increased the blood-brain barrier permeability in rat MCAO models. This splenic response could be blocked by MSCs implantation, which suggested a novel immunomodulatory approach to mediate neuronal protection [75]. In the previous studies, MSCs were shown to interact with innate and adaptive immune cells and express immunomodulatory cytokines [76]. The transplanted MSCs could decrease the number of inflammatory cells released by spleen [77], upregulate anti-inflammatory cytokine IL-10 and downregulate proinflammatory cytokine IL-1β [78]. These reports supported the concept that hUC-MSCs participated in the early tissue remodeling process of cerebral infarction partly by controlling inflammatory response.

In summary, hUC-MSCs administration may aid in reducing the long-term impact of stroke via multiple mechanisms that still have to be fully elucidated [79]. Plenty of transplanted cells migrated to lung and liver, or became apoptotic eventually in host brain. Few originally delivered hUC-MSCs could retain residency and differentiate into active neural cells. Therefore, the indirect paracrine mechanisms, including the secretion of neurotrophic and anti-apoptotic factors, the anti-inflammatory ability, the capacity to induce neurogenesis and angiogenesis, are more likely to accelerate neurorestoration [80]. Potential therapeutic mechanisms of hUC-MSCs implantation for ischemic stroke are shown as Fig. 1.

The possible risks of the treatment

Despite the special biological characteristics and enormous potential benefits in application of hUC-MSCs, there are still some possible risks exist in hUC-MSCs based clinical

therapy. One of the unresolved issues would be related to the application of allogeneic cells transplantation [81]. As previously discussed, hUC-MSCs can be used as a potential valid alternative to stroke, as they could prevent allograft rejection. However, the precise mechanism responsible for this effect and whether it will persist long-term in clinical treatment remain to be determined by further studies. In this case, allogeneic rather than autologous cells may expose the recipients to graft-versus-host diseases, which would complicate the treatment. Another potential risk is the inducement of infection [75]. Delivering MSCs to cerebrospinal fluid or ischemic cortex would likely elevate the probability of infection in comparison with intravenous implantation.

In addition, there are more and more evidences to show that MSCs have the potential to migrate into tumor and promote tumor cell proliferation, invasion and metastasis [82,83]. Using an nude mice model of colon cancer, orthotopic transplantation of cancer cells mixed with MSCs resulted in greater tumor weight and significantly lower survival rate than delivery of KM12SM cells alone did [84]. The mechanisms may lie on suppressing the antitumor immune response, directly providing nourishment to tumor cells and inhibiting apoptosis of tumor cells [85,86]. Although hUC-MSCs did not produce tumors *in vitro* experiments, further researches are necessary to evaluate their tumorigenic possibilities. And there should be more systematic reports about the complications in hUC-MSCs-based treatment to evaluate the benefit-to-risk ratio of cellular therapy in ischemic stroke.

Conclusions

Human umbilical cord mesenchymal stem cells are collected from Wharton's jelly and perivascular region in the umbilical cord, which is generally considered to be a "leftover" after childbirth and can be easily gained without ethical controversy. hUC-MSCs have the unique property of self-renewing and proliferation, and no host response or tumorigenesis is induced by hUC-MSCs in animal experiments. Researchful evidence in preclinical models indicates that hUC-MSCs transplantation therapy is promising to achieve neural repair and protection.

Clinical trials on MSCs transplantation for stroke are currently ongoing. In patients with ischemic stroke, intravenous transplantation of autologous MSCs derived from BM could improve functional recovery without safety concerns for death, stroke recurrence, or serious adverse events up to 1 year [87]. Lee *et al.* reported a long-term follow-up study for 5 years and observed no evidence for venous thromboembolism, systemic malignancy or systemic infection in any of the patients following MSCs

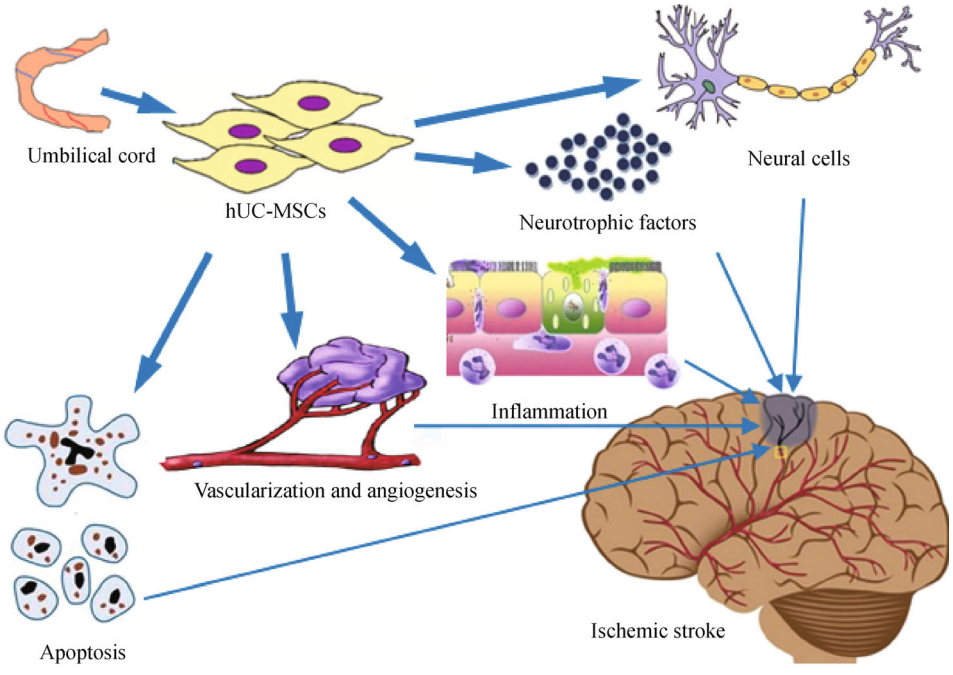

Fig. 1 Potential therapeutic mechanisms of hUC-MSCs implantation for ischemic stroke: (1) replacement of damaged nervous cells; (2) promotion of endogenous neural cells; (3) secretion of neurotrophic factors; (4) induction of vascularization and angiogenesis; (5) reduction of apoptosis; (6) prevention of inflammatory effect.

infusion [88]. In 2011, the first report of hUC-MSCs in a human clinical application is reported [89]. hUC-MSCs were found to have superior proliferative potential and more suppressive effects on peripheral blood mononuclear cell proliferation compared with bone marrow MSCs. However, currently there is no clinical trial which has been applied on hUC-MSCs transplantation for ischemic stroke (http://clinicaltrials.gov). It's too early to decide that hUC-MSCs administration can be clinically developed to promote recovery after stroke. The animal model can not simulate all aspects of pathological process in stroke, and the behavior changes after cell transplantation in animal models may only partly reflect the situation in patients. Moreover, several uncertain problems, such as the culture conditions, inducement of allograft rejection and potential tumorigenicity, are the choke points in this cellular therapy. More preclinical researches and clinical studies are needed before these cells can be used as a routinely applied clinical treatment.

Acknowledgements

This study was supported by National Natural Science Foundation of China (No. 81273751), the Project for Key Laboratories in Hunan Province — the Prophylaxis and Treatment for Nervous System Diseases with Combination of TCM and Western Medicine (No. 2014XNFZ01) and the Innovative Scientific Research Projects of Graduate Students in Hunan Province in 2014 (No. CX2014B348).

Compliance with ethics guidelines

Yingchen Li, Guoheng Hu, and Qilai Cheng have declared that no conflict of interest exists, and the authors have no financial relationships to disclose. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

References

1. Zhang L, Li Y, Zhang C, Chopp M, Gosiewska A, Hong K. Delayed administration of human umbilical tissue-derived cells improved neurological functional recovery in a rodent model of focal ischemia. Stroke 2011; 42(5): 1437–1444
2. McGuckin CP, Jurga M, Miller AM, Sarnowska A, Wiedner M, Boyle NT, Lynch MA, Jablonska A, Drela K, Lukomska B, Domanska-Janik K, Kenner L, Moriggl R, Degoul O, Perruisseau-Carrier C, Forraz N. Ischemic brain injury: a consortium analysis of key factors involved in mesenchymal stem cell-mediated inflammatory reduction. Arch Biochem Biophys 2013; 534(1–2): 88–97
3. Blum B, Benvenisty N. The tumorigenicity of human embryonic stem cells. Adv Cancer Res 2008; 100: 133–158
4. Cai J, Li W, Su H, Qin D, Yang J, Zhu F, Xu J, He W, Guo X, Labuda K, Peterbauer A, Wolbank S, Zhong M, Li Z, Wu W, So KF, Redl H, Zeng L, Esteban MA, Pei D. Generation of human induced pluripotent stem cells from umbilical cord matrix and amniotic membrane mesenchymal cells. J Biol Chem 2010; 285(15): 11227–11234
5. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006; 126(4): 663–676
6. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284(5411): 143–147
7. Li C, Li B, Dong Z, Gao L, He X, Liao L, Hu C, Wang Q, Jin Y. Lipopolysaccharide differentially affects the osteogenic differentiation of periodontal ligament stem cells and bone marrow mesenchymal stem cells through Toll-like receptor 4 mediated nuclear factor κB pathway. Stem Cell Res Ther 2014; 5(3): 67
8. Li D, Wang C, Shan W. Human amnion tissue injected with human umbilical cord mesenchymal stem cells repairs damaged sciatic nerves in rats. Neural Regen Res 2012; 7(23): 1771–1778
9. Sarugaser R, Lickorish D, Baksh D, Hosseini MM, Davies JE. Human umbilical cord perivascular (HUCPV) cells: a source of mesenchymal progenitors. Stem Cells 2005; 23(2): 220–229
10. Guo J, Fan HH, Qian YX. IFN-γ can promote the immunosuppressive capacity of human umbilical cord mesenchymal stem cells by expression of indoleamine 2,3-dioxygenase. J Diagn Concepts Pract 2010; 9(3): 181–185
11. Li DR, Cai JH. Methods of isolation, expansion, differentiating induction and preservation of human umbilical cord mesenchymal stem cells. Chin Med J (Engl) 2012; 125(24): 4504–4510
12. Kadam SS, Tiwari S, Bhonde RR. Simultaneous isolation of vascular endothelial cells and mesenchymal stem cells from the human umbilical cord. In Vitro Cell Dev Biol Anim 2009; 45(1–2): 23–27
13. Dong M, Chen J, Ma YQ. Efficient method for isolation of human umbilical cord mesenchymal stem cells. Chin J Tissue Eng Res (Zhongguo Zu Zhi Gong Cheng Yan Jiu) 2012; 16(45): 8406–8412 (in Chinese)
14. Liu SP, Ding DC, Wang HJ, Su CY, Lin SZ, Li H, Shyu WC. Nonsenescent Hsp27-upregulated MSCs implantation promotes neuroplasticity in stroke model. Cell Transplant 2010; 19(10): 1261–1279
15. Sensebé L, Krampera M, Schrezenmeier H, Bourin P, Giordano R. Mesenchymal stem cells for clinical application. Vox Sang 2010; 98 (2): 93–107
16. Li JJ, Li D, Ju XL, Liu WB. Umbilical cord-derived mesenchymal stem cells retain immunomodulatory and anti-oxidative activities after neural induction. Neural Regen Res 2012; 7(34): 2663–2672
17. Arufe MC, De la Fuente A, Fuentes I, Toro FJ, Blanco FJ.Umbilical cord as a mesenchymal stem cell source for treating joint pathologies. World J Orthop 2011; 2(6): 43–50
18. Liu L, Zhao X, Li P, Zhao G, Wang Y, Hu Y, Hou Y. A novel way to isolate MSCs from umbilical cords. Eur J Immunol 2012; 42(8): 2190–2193
19. Malgieri A, Kantzari E, Patrizi MP, Gambardella S. Bone marrow and umbilical cord blood human mesenchymal stem cells: state of the art. Int J Clin Exp Med 2010; 3(4): 248–269
20. Xu LX, Cao YB, Liu ZY, Wu YM, Wang ZH, Yan B, Da WM, Wu

XX. Transplantation of haploidentical-hematopoietic stem cells combined with two kind of third part cells for chronic aplastic anemia: one case report. J Exp Hematol (Zhongguo Shi Yan Xue Ye Xue Za Zhi) 2013; 21(6): 1522–1525 (in Chinese)
21. Gu W, Gu J. Homing mechanism of umbilical cord mesenchymal stem cells. Chin J Tissue Eng Res (Zhongguo Zu Zhi Gong Cheng Yan Jiu) 2013; 17(6): 1135–1140 (in Chinese)
22. Li DR, Cai JH. Methods of isolation, expansion, differentiating induction and preservation of human umbilical cord mesenchymal stem cells. Chin Med J (Engl) 2012; 125(24): 4504–4510
23. Hass R, Kasper C, Böhm S, Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): a comparison of adult and neonatal tissue-derived MSC. Cell Commun Signal 2011; 9(1): 12
24. Guo J, Yang J, Cao G, Fan H, Guo C, Ma YE, Qian Y, Chen L, Li X, Chang C. Xenogeneic immunosuppression of human umbilical cord mesenchymal stem cells in a major histocompatibility complex-mismatched allogeneic acute graft-versus-host disease murine model. Eur J Haematol 2011; 87(3): 235–243
25. Wang D, Chen K, Du WT, Han ZB, Ren H, Chi Y, Yang SG, Bayard F, Zhu D, Han ZC. $CD14^{+}$ monocytes promote the immunosuppressive effect of humanumbilical cord matrix stem cells. Exp Cell Res 2010; 316(15): 2414–2423
26. Das M, Sundell IB, Koka PS. Adult mesenchymal stem cells and their potency in the cell-based therapy. J Stem Cells 2013; 8(1): 1–16
27. Atoui R, Shum-Tim D, Chiu RC. Myocardial regenerative therapy: immunologic basis for the potential "universal donor cells". Ann Thorac Surg 2008; 86(1): 327–334
28. Patel DM, Shah J, Srivastava AS. Therapeutic potential of mesenchymal stem cells in regenerative medicine. Stem Cells Int 2013; 2013: 496218
29. Liu GY, Xu Y, Li Y, Wang LH, Liu YJ, Zhu D. Secreted galectin-3 as a possible biomarker for the immunomodulatory potential of human umbilical cord mesenchymal stromal cells. Cytotherapy 2013; 15(10): 1208–1217
30. Wang D, Ji YR, Chen K, Du WT, Yang ZX, Han ZB, Chi Y, Liang L, Bayard F, Han ZC. IL-6 production stimulated by CD14(+) monocytes-paracrined IL-1β does not contribute to the immunosuppressive activity of human umbilical cord mesenchymal stem cells. Cell Physiol Biochem 2012; 29(3–4): 551–560
31. Wu CC, Wu TC, Liu FL, Sytwu HK, Chang DM. TNF-α inhibitor reverse the effects of human umbilical cord-derived stem cells on experimental arthritis by increasing immunosuppression. Cell Immunol 2012; 273(1): 30–40
32. Liu GY, Xu Y, Li Y, Wang LH, Liu YJ, Zhu D. Secreted galectin-3 as a possible biomarker for the immunomodulatory potential of human umbilical cord mesenchymal stromal cells. Cytotherapy 2013; 15(10): 1208–1217
33. Greco SJ, Rameshwar P. Mesenchymal stem cells in drug/gene delivery: implications for cell therapy. Ther Deliv 2012; 3(8): 997–1004
34. Fong CY, Chak LL, Biswas A, Tan JH, Gauthaman K, Chan WK, Bongso A. Human Wharton's jelly stem cells have unique transcriptome profiles compared to human embryonic stem cells and other mesenchymal stem cells. Stem Cell Rev 2011; 7(1): 1–16
35. Liu X, Ye R, Yan T, Yu SP, Wei L, Xu G, Fan X, Jiang Y, Stetler RA, Liu G, Chen J. Cell based therapies for ischemic stroke: from basic science to bedside. Prog Neurobiol 2014; 115: 92–115
36. Liao W, Xie J, Zhong J, Liu Y, Du L, Zhou B, Xu J, Liu P, Yang S, Wang J, Han Z, Han ZC. Therapeutic effect of human umbilical cord multipotent mesenchymal stromal cells in a rat model of stroke. Transplantation 2009; 87(3): 350–359
37. Lin YC, Ko TL, Shih YH, Lin MY, Fu TW, Hsiao HS, Hsu JY, Fu YS. Human umbilical mesenchymal stem cells promote recovery after ischemic stroke. Stroke 2011; 42(7): 2045–2053
38. Weise G, Lorenz M, Pösel C, Maria Riegelsberger U, Störbeck V, Kamprad M, Kranz A, Wagner DC, Boltze J. Transplantation of cryopreserved human umbilical cord blood mononuclear cells does not induce sustained recovery after experimental stroke in spontaneously hypertensive rats. J Cereb Blood Flow Metab 2014; 34(1): e1–e9
39. Pellegrini L, Bennis Y, Guillet B, Velly L, Bruder N, Pisano P. Cell therapy for stroke: from myth to reality. Rev Neurol (Paris) 2013; 169(4): 291–306 (in French)
40. Lindvall O, Kokaia Z. Stem cell research in stroke: how far from the clinic? Stroke 2011; 42(8): 2369–2375
41. Xia G, Hong X, Chen X, Lan F, Zhang G, Liao L. Intracerebral transplantation of mesenchymal stem cells derived from human umbilical cord blood alleviates hypoxic ischemic brain injury in rat neonates. J Perinat Med 2010; 38(2): 215–221
42. Messerli M, Wagner A, Sager R, Mueller M, Baumann M, Surbek DV, Schoeberlein A. Stem cells from umbilical cord Wharton's jelly from preterm birth have neuroglial differentiation potential. Reprod Sci 2013; 20(12): 1455–1464
43. Yan M, Sun M, Zhou Y, Wang W, He Z, Tang D, Lu S, Wang X, Li S, Wang W, Li H. Conversion of human umbilical cord mesenchymal stem cells in Wharton's jelly to dopamine neurons mediated by the Lmx1a and neurturin *in vitro*: potential therapeutic application for Parkinson's disease in a rhesus monkey model. PLoS ONE 2013; 8(5): e64000
44. Lees JS, Sena ES, Egan KJ, Antonic A, Koblar SA, Howells DW, Macleod MR. Stem cell-based therapy for experimental stroke: a systematic review and meta-analysis. Int J Stroke 2012; 7(7): 582–588
45. Weiss ML, Medicetty S, Bledsoe AR, Rachakatla RS, Choi M, Merchav S, Luo Y, Rao MS, Velagaleti G, Troyer D. Human umbilical cord matrix stem cells: preliminary characterization and effect of transplantation in a rodent model of Parkinson's disease. Stem Cells 2006; 24(3): 781–792
46. Liu XS, Chopp M, Wang XL, Zhang L, Hozeska-Solgot A, Tang T, Kassis H, Zhang RL, Chen C, Xu J, Zhang ZG. MicroRNA-17-92 cluster mediates the proliferation and survival of neural progenitor cells after stroke. J Biol Chem 2013; 288(18): 12478–12488
47. Tornero D, Wattananit S, Grønning Madsen M, Koch P, Wood J, Tatarishvili J, Mine Y, Ge R, Monni E, Devaraju K, Hevner RF, Brüstle O, Lindvall O, Kokaia Z. Human induced pluripotent stem cell-derived cortical neurons integrate in stroke-injured cortex and improve functional recovery. Brain 2013; 136(12): 3561–3577
48. Martí-Fàbregas J, Romaguera-Ros M, Gómez-Pinedo U, Martínez-Ramírez S, Jiménez-Xarrié E, Marín R, Martí-Vilalta JL, García-Verdugo JM. Proliferation in the human ipsilateral subventricular zone after ischemic stroke. Neurology 2010; 74(5): 357–365
49. Jin K, Wang X, Xie L, Mao XO, Greenberg DA. Transgenic ablation

of doublecortin-expressing cells suppresses adult neurogenesis and worsens stroke outcome in mice. Proc Natl Acad Sci USA 2010; 107 (17): 7993–7998

50. Raber J, Fan Y, Matsumori Y, Liu Z, Weinstein PR, Fike JR, Liu J. Irradiation attenuates neurogenesis and exacerbates ischemia-induced deficits. Ann Neurol 2004; 55(3): 381–389
51. Dibajnia P, Morshead CM. Role of neural precursor cells in promoting repair following stroke. Acta Pharmacol Sin 2013; 34(1): 78–90
52. Liu C, Sun J. Potential application of hydrolyzed fish collagen for inducing the multidirectional differentiation of rat bone marrow mesenchymal stem cells. Biomacromolecules 2014; 15(1): 436–443
53. Kim SS, Yoo SW, Park TS, Ahn SC, Jeong HS, Kim JW, Chang DY, Cho KG, Kim SU, Huh Y, Lee JE, Lee SY, Lee YD, Suh-Kim H. Neural induction with neurogenin1 increases the therapeutic effects of mesenchymal stem cells in the ischemic brain. Stem Cells 2008; 26(9): 2217–2228
54. Seo JH, Cho SR. Neurorestoration induced by mesenchymal stem cells: potential therapeutic mechanisms for clinical trials. Yonsei Med J 2012; 53(6): 1059–1067
55. Xu W, Wang X, Xu G, Guo J. Light-induced retinal injury enhanced neurotrophins secretion and neurotrophic effect of mesenchymal stem cells *in vitro*. Arq Bras Oftalmol 2013; 76(2): 105–110
56. Choi M, Lee HS, Naidansaren P, Kim HK, O E, Cha JH, Ahn HY, Yang PI, Shin JC, Joe YA. Proangiogenic features of Wharton's jelly-derived mesenchymal stromal/stem cells and their ability to form functional vessels. Int J Biochem Cell Biol 2013; 45(3): 560–570
57. Alder J, Kramer BC, Hoskin C, Thakker-Varia S. Brain-derived neurotrophic factor produced by human umbilical tissue-derived cells is required for its effect on hippocampal dendritic differentiation. Dev Neurobiol 2012; 72(6): 755–765
58. Ribeiro CA, Fraga JS, Grãos M, Neves NM, Reis RL, Gimble JM, Sousa N, Salgado AJ. The secretome of stem cells isolated from the adipose tissue and Wharton jelly acts differently on central nervous system derived cell populations. Stem Cell Res Ther 2012; 3(3): 18
59. Qu R, Li Y, Gao Q, Shen L, Zhang J, Liu Z, Chen X, Chopp M. Neurotrophic and growth factor gene expression profiling of mouse bone marrow stromal cells induced by ischemic brain extracts. Neuropathology 2007; 27(4): 355–363
60. Verina T, Fatemi A, Johnston MV, Comi AM. Pluripotent possibilities: human umbilical cord blood cell treatment after neonatal brain injury. Pediatr Neurol 2013; 48(5): 346–354
61. Liu XL, Zhang W, Tang SJ. Intracranial transplantation of human adipose-derived stem cells promotes the expression of neurotrophic factors and nerve repair in rats of cerebral ischemia-reperfusion injury. Int J Clin Exp Pathol 2014; 7(1): 174–183
62. Petrova ES. The use of stem cells to stimulate regeneration of damaged nerve. Tsitologiia 2012; 54(7): 525–540 (in Russian)
63. Liu Z, Huang D, Zhang M, Chen Z, Jin J, Huang S, Zhang Z, Wang Z, Chen L, Chen L, Xu Y. Cocaine- and amphetamine-regulated transcript promotes the differentiation of mouse bone marrow-derived mesenchymal stem cells into neural cells. BMC Neurosci 2011; 12: 67
64. Wang LL, Chen D, Lee J, Gu X, Alaaeddine G, Li J, Wei L, Yu SP. Mobilization of endogenous bone marrow derived endothelial progenitor cells and therapeutic potential of parathyroid hormone after ischemic stroke in mice. PLoS ONE 2014; 9(2): e87284
65. Liu XL, Zhang W, Tang SJ. Intracranial transplantation of human adipose-derived stem cells promotes the expression of neurotrophic factors and nerve repair in rats of cerebral ischemia-reperfusion injury. Int J Clin Exp Pathol 2014; 7(1): 174–183
66. Akimoto K, Kimura K, Nagano M, Takano S, To'a Salazar G, Yamashita T, Ohneda O. Umbilical cord blood-derived mesenchymal stem cells inhibit, but adipose tissue-derived mesenchymal stem cells promote, glioblastoma multiforme proliferation. Stem Cells Dev 2013; 22(9): 1370–1386
67. Zhai XD, Chen ZY, Leng XF, Wang YJ, Chen Lu, Jiang S. Treat flap ischemia-reperfusion injury by local transplanting human umbilical cord mesenchymal stem cells. Chin J Plast Surg (Zhonghua Zheng Xing Wai Ke Za Zhi) 2012; 28(3): 203–207 (in Chinese)
68. Kim WR, Sun W. Programmed cell death during postnatal development of the rodent nervous system. Dev Growth Differ 2011; 53(2): 225–235
69. Lin WY, Chang YC, Ho CJ, Huang CC. Ischemic preconditioning reduces neurovascular damage after hypoxia-ischemia via the cellular inhibitor of apoptosis 1 in neonatal brain. Stroke 2013; 44 (1): 162–169
70. Huang W, Mo X, Qin C, Zheng J, Liang Z, Zhang C. Transplantation of differentiated bone marrow stromal cells promotes motor functional recovery in rats with stroke. Neurol Res 2013; 35(3): 320–328
71. Scheibe F, Klein O, Klose J, Priller J. Mesenchymal stromal cells rescue cortical neurons from apoptotic cell death in an *in vitro* model of cerebral ischemia. Cell Mol Neurobiol 2012; 32(4): 567–576
72. Scuteri A, Ravasi M, Pasini S, Bossi M, Tredici G. Mesenchymal stem cells support dorsal root ganglion neurons survival by inhibiting the metalloproteinase pathway. Neuroscience 2011; 172: 12–19
73. Jin R, Yang G, Li G. Inflammatory mechanisms in ischemic stroke: role of inflammatory cells. J Leukoc Biol 2010; 87(5): 779–789
74. Carroll J. Human cord blood for the hypoxic-ischemic neonate. Pediatr Res 2012; 71(4 Pt 2): 459–463
75. Womble TA, Green S, Shahaduzzaman M, Grieco J, Sanberg PR, Pennypacker KR, Willing AE. Monocytes are essential for the neuroprotective effect of human cord blood cells following middle cerebral artery occlusion in rat. Mol Cell Neurosci 2014; 59: 76–84
76. Bickels J, Weinstein T, Robinson D, Nevo Z. Common skeletal growth retardation disorders resulting from abnormalities within the mesenchymal stem cells reservoirs in the epiphyseal organs pertaining to the long bones. J Pediatr Endocrinol Metab 2010; 23 (11): 1107–1122
77. Leonardo CC, Hall AA, Collier LA, Ajmo CT Jr, Willing AE, Pennypacker KR. Human umbilical cord blood cell therapy blocks the morphological change and recruitment of CD11b-expressing, isolectin-binding proinflammatory cells after middle cerebral artery occlusion. J Neurosci Res 2010; 88(6): 1213–1222
78. Seo JH, Jang IK, Kim HB, Yang MS, Lee JE, Kim HE, Eom YW, Lee DH, Yu JH, Kim JY, Kim HO, Cho SR. Immunomodulation from intravenous transplantation of mesenchymal stem cells promotes functional recovery in spinal cord injured rats. Cell Med 2011; 2(2): 55–67
79. Eckert MA, Vu Q, Xie K, Yu J, Liao W, Cramer SC, Zhao W.

Evidence for high translational potential of mesenchymal stromal cell therapy to improve recovery from ischemic stroke. J Cereb Blood Flow Metab 2013; 33(9): 1322–1334

80. Petrie Aronin CE, Tuan RS. Therapeutic potential of the immunomodulatory activities of adult mesenchymal stem cells. Birth Defects Res C Embryo Today 2010; 90(1): 67–74
81. Carroll JE, Mays RW. Update on stem cell therapy for cerebral palsy. Expert Opin Biol Ther 2011; 11(4): 463–471
82. Wang D, Wang S, Shi C. Update on cancer related issues of mesenchymal stem cell-based therapies. Curr Stem Cell Res Ther 2012; 7(5): 370–380
83. Lua I, James D, Wang J, Wang KS, Asahina K. Mesodermal mesenchymal cells give rise to myofibroblasts, but not epithelial cells, in mouse liver injury. Hepatology 2014; 60(1): 311–322
84. Lin JT, Wang JY, Chen MK, Chen HC, Chang TH, Su BW, Chang PJ. Colon cancer mesenchymal stem cells modulate the tumorigenicity of colon cancer through interleukin 6. Exp Cell Res 2013; 319(14): 2216–2229
85. Rameshwar P. Would cancer stem cells affect the future investment in stem cell therapy. World J Exp Med 2012; 2(2): 26–29
86. Lin G, Yang R, Banie L, Wang G, Ning H, Li LC, Lue TF, Lin CS. Effects of transplantation of adipose tissue-derived stem cells on prostate tumor. Prostate 2010; 70(10): 1066–1073
87. Honmou O, Houkin K, Matsunaga T, Niitsu Y, Ishiai S, Onodera R, Waxman SG, Kocsis JD. Intravenous administration of auto serum-expanded autologous mesenchymal stem cells in stroke. Brain 2011; 134(6): 1790–1807
88. Lee JS, Hong JM, Moon GJ, Lee PH, Ahn YH, Bang OY; STARTING collaborators. A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. Stem Cells 2010; 28(6): 1099–1106
89. Chen GH, Yang T, Tian H, Qiao M, Liu HW, Fu CC, Miao M, Jin ZM, Tang XW, Han Y, He GS, Zhang XH, Ma X, Chen F, Hu XH, Xue SL, Wang Y, Qiu HY, Sun AN, Chen ZZ, Wu DP. Clinical study of umbilical cord-derived mesenchymal stem cells for treatment of nineteen patients with steroid-resistant severe acute graft-versus-host disease. Chin J Hematol (Zhonghua Xue Ye Xue Za Zhi) 2012; 33(4): 303–306 (in Chinese)