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Diverse actions of cord blood cell therapy for hypoxic-ischemic encephalopathy

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Abstract Perinatal hypoxic-ischemic encephalopathy (HIE) is a major cause of neonatal death and permanent neurological deficits. However, effective treatments have not yet been established, except therapeutic hypothermia, which is not effective for severe HIE; therefore, developing a novel therapy for HIE is of the utmost importance. Stem cell therapy has recently been identified as a novel therapy for HIE. Among the various stem cell sources, ethical hurdles can be avoided by using stem cells that originate from non-embryonic or non-neural tissues, such as umbilical cord blood cells (UCBCs), which are readily available and can be exploited for autologous transplantations. Human UCBs are a rich source of stem and progenitor cells. Many recent studies have reported the treatment effect of UCBCs. Additionally, phase I clinical trials have already been conducted, showing this therapy's safety and feasibility. One advantage of stem cell therapies, including UCBC administration, is that they exert treatment effects through multifaceted mechanisms. According to the findings of several publications, replacement of lost cells, namely, engraftment and differentiation into neuronal cells, is not likely to be the main mechanism. However, the association between UCBCs and various mechanism of action, such as neurogenesis, angiogenesis, and anti-inflammation, has been suggested in many studies, and most mechanisms are due to growth factors secreted from UCBCs. These diverse actions of UCBC treatment are expected to exert a substantial effect on HIE, which has a complex injury mechanism.

Key words brain injury, growth factor, neonate, stem cells.

Introduction

The global neonatal mortality rate has improved dramatically in the last 30 years.[1] However, the incidence of cerebral palsy has not decreased at all.[2] Perinatal hypoxic-ischemic encephalopathy (HIE) is the main cause of cerebral palsy, with an incidence of 1.3–1.7 per 1,000 births worldwide.[3] Cerebral palsy is a lifelong condition and can be a huge burden on the patient and his/her family. The only effective treatment for perinatal HIE is therapeutic hypothermia;[4,5] however, this treatment's effects are limited and, additionally, the lifetime cost of medical care and training/education per patient is approximately 1 million US dollars (100 million Japanese yen),[6,7] which is a tremendous financial burden. Generally, the Number-Needed-to-Treat (NNT) is 9, i.e., nine patients are needed to be treated in order to save one patient from death or severe disability at 18 months of age.[8] There is an urgent need, therefore, to develop new treatments for this perinatal brain injury.

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Stem cell therapy is expected to become a novel therapy for central nervous system (CNS) diseases.[9] Stem cells are derived from various tissue types and defined by their self-renewal and multipotency properties. So far, various types of stem cells, including embryonic stem (ES) cells, neural stem/progenitor cells (NSPCs), bone marrow stromal cells, umbilical cord blood (UCB) stem cells, and induced pluripotent stem (iPS) cells, have been used in the research of stem cell treatments for CNS disorders.[10] Among the various stem cell sources, NSPCs were most widely used in early studies on CNS diseases. In previous studies, we demonstrated that intracerebroventricular injections of NSPCs with chondroitinase ABC, which digests glycosaminoglycan chains on chondroitin sulfate proteoglycans, reduced the degree of brain injury in a rat neonatal HI model.[10,11] However, hurdles remain concerning the clinical application of NSPCs, such as ethical issues regarding collecting these stem cells from fetuses and safety concerns associated with their intracerebral administration.[12]

In contrast, umbilical cord blood cells (UCBCs) are readily available and there are no ethical issues regarding their collection. Human UCB is a rich source of stem and progenitor cells, and the treatment effect of UCBCs have recently been evaluated in animal models of neonatal HI in over 20 studies. In most of these preclinical studies, UCBCs were administered

systemically, and many of them showed beneficial effects.[13] Furthermore, phase I clinical trials have already been conducted showing the safety and feasibility of intravenous administration of autologous UCBCs.[14,15]

One advantage of stem cell therapy, including UCBC administration, is that the cells exert treatment effects through many mechanisms. Stem cells produce a large number of growth factors that act on many mechanisms to produce therapeutic effects,[16–18] whereby, even if only a few cells among systemically infused cells migrate and engraft into the brain,[19] the treatment can still be effective. This multitude of action mechanisms is a major advantage in UCBC treatment. Downstream signaling pathways leading to brain injury after insult in HIE are complicated, like "whirling tides", in which multiple signaling pathways converge, diverge, and make feedback loops to upstream of their own pathways and/or other pathways, eventually revolving again and again.[20] Blocking a single cascade will result in other cascades being compensatory and leading to no, or only a marginal, therapeutic effect. To be effective against such a complex injury mechanism would require treatments that work on many mechanisms/cascades simultaneously.

This article reviews UCBC treatments for HIE, with a focus on the mechanisms, and shows the multifaceted nature of the treatment (Fig 1).

Mechanism of UCBC treatments for HIE

Engraftment of UCBCs and differentiation into neuronal cells in the lesion

Several studies have evaluated UCBC engraftment in the brain. Meier *et al*. were the first to report UCBC treatment in an animal model of perinatal HIE.[21] These authors injected mononuclear cells (MNCs) from human UCB (hUCB) intraperitoneally into HIE rats and identified the administered human cells by the immunohistochemical detection of human-specific human leukocyte antigen-DR α-chain surface antigens. Meier *et al*. also demonstrated that many transplanted hUCBCs migrated to the damaged brain lesion 3 days after the injection and were still present 2 weeks after the transplantation. However, these cells did not overlap with neural markers, such as glial fibrillary acidic protein (GFAP; astrocyte marker), neurofilament-68, or synaptophysin (neuronal markers), indicating that they did not differentiate into neural cells.

In contrast, in our observation,[19] only a few cells were detected in HIE rat brains following administration. We intraperitoneally administered MNCs cultured with growth factors, which were derived from the UCB of green fluorescent protein (GFP)-transgenic rats and then evaluated the treatment effect and engraftment in the brain. Although the treatment effects were verified histologically and functionally, the GFP-positive cells were hardly detectable in the brain (0.0057% of injected cells) 9 days after administration. Additionally, 60% of GFP-positive cells in the brain were Iba1-positive, and none of these was positive for neuronal markers (neurogenic differentiation factor or doublecortin [DCX]). Pimentel-Coelho *et al*. also traced intraperitoneal injected MNCs from hUCB labeled with CellTrace, and evaluated the cells using an anti-human nuclei antibody; however, only a few cells were found in the cortex or the striatum using either method.[22] Yasuhara *et al*.[23] evaluated intravenously injected hUCB-MNCs with an anti-human nuclei antibody 14 days after transplantation. Only sporadic surviving hUCB-MNCs (approx. 2–25 cells per brain) were detected, although functional recovery was observed in behavioral tests. In Bea *et al*.'s study,[24] many human nuclei (HN)-positive cells (transplanted cells) containing NSPCs marker (Nestin) or the immature neuronal marker (DCX) were found in the periventricular region on the side of the insult 1 week after the intravenous hUCB-MNC transplantation. However, these positive cells decreased dramatically over time, and at 3 and 10 weeks after treatment, few HN-positive cells were seen in the same region.

Taken together, although some publications showed significant engraftment of the transplanted UCBCs, most reports only described a few cells, and surviving cells are likely to decrease over time. Considering the long-term therapeutic effects,[24] the direct effect derived from engrafted UCBCs is likely to contribute little to the overall therapeutic effect.

Neurogenesis

Neurogenesis occurs in the subventricular zone (SVZ) and the hippocampus dentate gyrus subgranular zone throughout life.[25,26] Wang *et al*.[27] in their study evaluated the effect of UCBC administration on endogenous neurogenesis in detail. In the SVZ, neurogenesis was enhanced 3 days after HI insult, but returned to the baseline approximately 7 days after HI. When hUCB-MNCs were transplanted intraventricularly, the neurogenesis was further enhanced, even 7 days after HI. They showed that the promoted neurogenesis was via the Sonic hedgehog (Shh) signaling pathway. Additionally, the same group revealed that UCBCs regulate the differentiation of endogenous neural stem cells after HIE, also via the Shh signaling pathway.[28]

Our study[19] showed that rat UCBCs ameliorated the number of proliferating cells (Ki67-positive cells) reduced by the insult in the ipsilateral hippocampus, 3 weeks after HIE. Also, in the SVZ, the number of proliferating cells generally increased due to the UCBCs.

In Bea *et al*.'s study,[24] the hUCB-MNC-treated group showed a higher number of DCX or Nestin-positive cells, with DCX and Nestin being immature neuronal and NSPCs markers, respectively, in the SVZ 1 week after the treatment. Moreover, these positive cells were spread and extended to the periventricular region, even in the striatum.

Although more studies are needed, the current body of evidence shows that systemic administration of UCBCs enhances endogenous neurogenesis after perinatal HIE.

Angiogenesis and cerebral blood flow

Vascular endothelial growth factor (VEGF) is up-regulated in the acute phase after HI injury.[29–31] VEGF-mediated

angiogenesis stimulated neural stem cell proliferation and differentiation.[32] Therefore, enhanced angiogenesis may produce further treatment effects against the brain injury.

Meier's group revealed that hUCBC application up-regulated VEGF mRNA expression, which has vasodilation and pro-angiogenic effects. Furthermore, UCBCs increased expression of the proteins that were associated with angiogenesis, Tie-2, and occluding.[33] As interleukin (IL)-8-mediated processes are essential for angiogenesis, endothelial cell proliferation, and capillary tube organization, Cho *et al.*[34] examined the role of IL-8 in hUCBC therapy. The administration of hUCB-MNCs 7 days after HIE up-regulated the gene expression of Cxcl2, the mouse IL-8 homolog.

We evaluated the treatment effect of $CD34^+$ cells in hUCB. We measured cerebral blood flow (CBF) with a laser speckle flowmetry imaging system (Omegazone, Omegawave Inc., Tokyo, Japan) and found that $CD34^+$ cell treatment significantly ameliorated the decreased CBF in the ischemic penumbra.[35] We also confirmed the effect of $CD34^+$ cell treatment on CBF using a the neonatal stroke model.[36]

Grandvuillemin *et al.*[37] evaluated cerebral capillary density using immunohistochemistry on postnatal day 14 (P14) and 12 weeks of age. It was significantly reduced by HI and significantly ameliorated by hUCB-MNC treatment. Additionally, CBF was examined using single-photon emission computed tomography. Although there was no significant difference at P14, the HI insult induced a significant decrease in CBF and UCBC treatment at 12 weeks of age, which produced a significant improvement.

However, the evaluation of capillary length using tomato-lectin staining 3 weeks after the insult in Nakanishi *et al.*'s report[19] indicated that HI insult reduced capillary length, but UCBC treatment did not improve it.

Most studies suggest that the administration of UCBCs ameliorated HI-induced vascular damage, reduced CBF, and enhanced angiogenesis. These beneficial UCBC effects seem reasonable as UCB contains endothelial progenitor cells that express $CD34^+$ and secrete VEGF.

Activated microglia

Microglia can be divided into two distinct types depending on their pro-inflammatory (M1) and anti-inflammatory (M2) status. Controlling or altering microglial polarity is one of the targets for treating CNS injuries, including HIE.[38,39] We evaluated activated microglia (M1) after intraperitoneal administration of hUCB-MNCs. Our study revealed that hUCBCs reduced the number of ED1- (an M1 microglia marker) positive cells significantly 24 h after HI insult.[40] Another study of ours involving rat UCBCs[19] confirmed that UCBC administration reduced ED1-positive cells and increased anti-Mannose receptor- (an M2 microglia marker) positive cells.

Other studies showed the same results as ours. Pimentel-Coelho *et al.*[22] demonstrated a reduced number of ED1-positive cells in the cortex 7 days after injection. Rosenkranz *et al.*[33] observed a reduction in the number of ED1-(CD68) positive cells 2 days after insult via immunoblotting and immunohistochemistry. Similarly, McDonald *et al.*[41] examined the effects of the intraperitoneal injection of subtypes of hUCBCs (i.e., MNCs, endothelial progenitor cells [EPCs], T regulatory cells [Tregs], and monocytes) in a rat model of neonatal HI. All the subtypes, apart from the monocytes, decreased the number of activated microglia that increased in the cerebral cortex after HI.

Park *et al.*[42,43] evaluated the synergistic effect of UCBC-derived mesenchymal stem cells (MSC) and hypothermia, which is the only established treatment for HIE at this moment. They transplanted UCBC-derived MSC into the ipsilateral ventricle. Although hypothermia could not exert an effect on activated microglia, the synergistic effect combined with these cells significantly suppressed it.

Penny *et al.*[44] examined the effects of the intraperitoneal administration of hUCB-MNCs 24 h after HI in a neonatal rat model and found that the cell treatment decreased HI-induced microglial activation. They also examined the treatment effect of multiple UCBC administrations: 24 h; 72 h; and 10 days after insult. Their results showed a significant increase in activated microglia in the HI group and a significant decrease in the 3-dose group, but not in the 1-dose group.

On the other hand, Bea *et al.*[24] showed that the number of Iba-1 (a pan-microglial marker)-positive cells, were significantly higher in the ipsilateral region of the hUCBC group than that of the vehicle group at 1 week after transplantation. However, as they did not examine the microglial polarity (M1 or M2), we do not know how the increased microglia affected the HIE rats.

Taken together, a large body of evidence shows that the systemic administration of UCBCs reduces the number of activated microglia, although there is a contradictory report. As microglia may become neuroprotective/restorative or detrimental after brain injury, careful evaluation is needed.

Astrogliosis

Pro-inflammatory cytokines and reactive species released in HI can trigger astrogliosis. Astrogliosis may initiate harmful effects on the developing brain after HI.[45]

Wasielewski *et al.*[46] showed that HI induced an acute inflammatory reaction with the activation of reactive astrogliosis and microglia. Astrocytes around the ischemic zone present an activated phenotype: a large soma and fewer processes after HI. UCBCs cause astrocytes to be a quiescent phenotype: a more elongated soma with long processes. Although a significant treatment effect of UCBCs did not affect astrogliosis at 7 days after HI, a huge, delayed decrease in astrogliosis was observed in the UCBC group 12 weeks after HI; Grandvuillemin *et al.*[37] also showed a significant and extended increase in astrogliosis 7 days and 12 weeks following HI insult.

Zhang *et al.* showed a similar effect in that UCBC administration in the lateral ventricle inhibited up-regulation of levels of GFAP (an astrocyte marker) labeling in the striatum 2 weeks after HI.[47]

Park *et al.*[42,43] also showed that the astrogliosis was more suppressed by the synergistic effect of UCBC-derived MSC

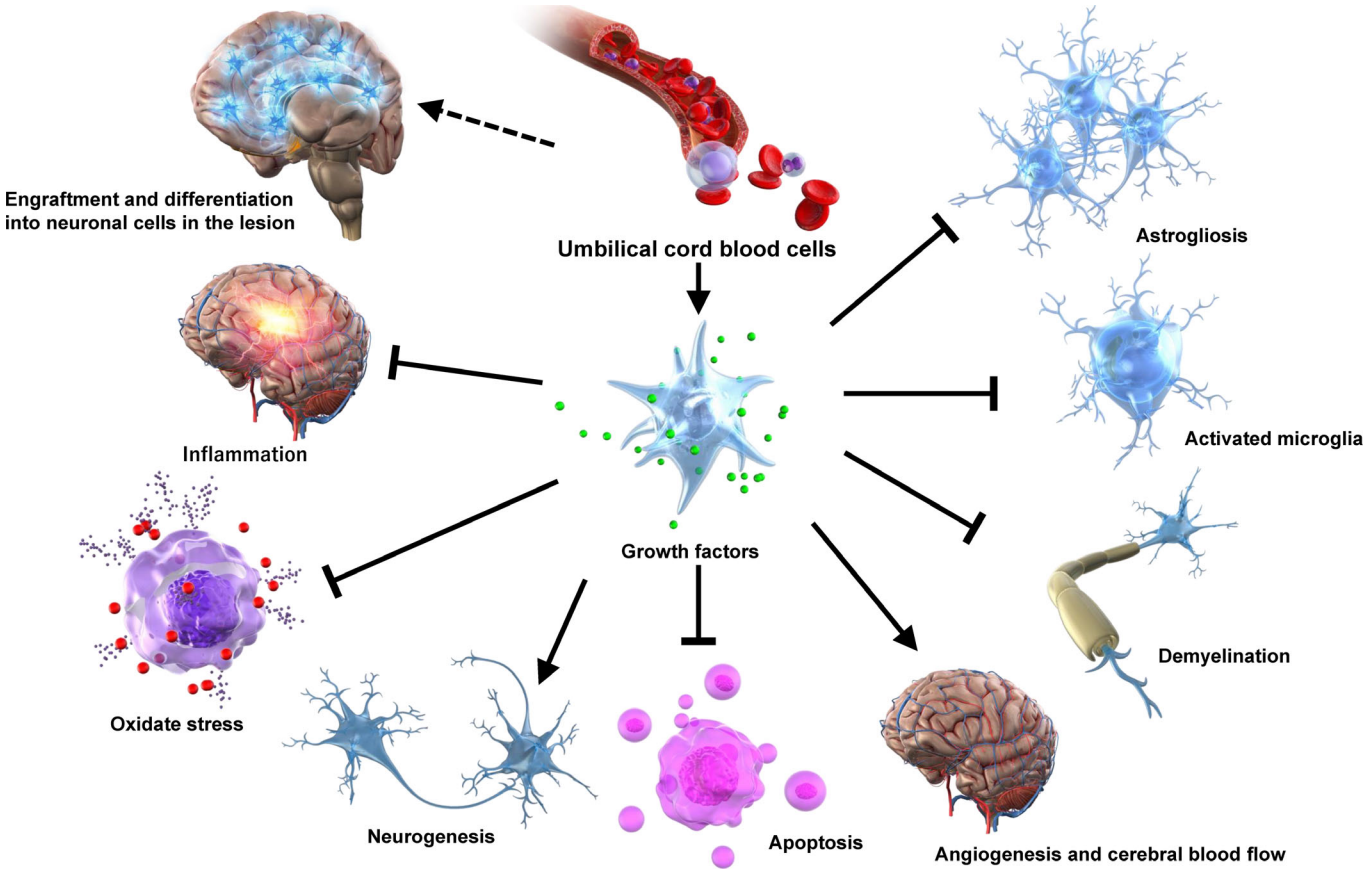

Fig. 1 Umbilical cord blood cell (UCBC) administration is a multifaceted treatment via diverse mechanisms for perinatal hypoxic-ischemic encephalopathy. Solid lines with arrowheads indicate an enhancement of the effect by UCBC administration. The dotted line with an arrowhead indicates the unlikely effect by UCBC administration. Solid lines with hammerheads indicate suppression of the effect by UCBC administration.

and hypothermia than by each individual treatment. Yu *et al.*[48] compared the effects of intravenous administration of hUCB-CD34$^+$ cells and MNCs in a rat model of neonatal HI and showed that either cell type inhibits GFAP expression.

Together, these studies show that hUCBC treatment exerts anti-astrogliosis effects.

Demyelination

In addition to ensuring axonal conduction velocity, intact myelin produces a neurotrophic substance to support axonal function and neuronal survival.[49] Therefore, demyelination can result in inadequate interconnections in the cortical regions.

Hypoxic-ischemic insult downregulated MBP (a myelin marker) expression in the cerebral cortex and corpus callosum 2 weeks after the insult; however, it was restored by USBC transplantation in the lateral ventricle.[47]

Inflammation (cytokines and chemokines)

Rosenkranz *et al.*[33] evaluated pro-inflammatory cytokines, IL-1α, IL-1β, and tumor necrosis factor α (TNFα) 2 days after HI insult in rats. All three pro-inflammatory cytokines were increased in the serum, and the administration of hUCBCs reduced these elevated serum levels of these cytokines. Park *et al.*[42,43] also showed the suppression of the elevated levels of these pro-inflammatory cytokines (IL-1α, IL-1β, Il-6, and TNFα) by the synergistic effect of UCBC-derived MSC and hypothermia, although hypothermia contributes to limited effects. Baba *et al.*[50] examined chemokine expression profiles in the brain extract of a neonatal HI mouse model. Intravenous infusion of hUCB-MNCs at 3 weeks after HI insult markedly increased CCL2, CCL12, CCL20 levels, and CX3CL1, which might relate to neural tissue regeneration. In addition to showing that all the cell types, except MNCs, reduced the Th1-mediated pro-inflammatory shift (i.e., increase in the ratio of Th1:Th2 cells) in the peripheral blood, McDonald *et al.*[41] showed that hUCB-MNCs, -EPCs, -Tregs, and -monocytes reduced the infiltration of CD4$^+$ T cells into the injured brain after HI.

Although evidence is limited to a few studies, UCBC administration is reported to reduce the elevated levels of pro-inflammatory cytokines/chemokines in the injured brain and peripheral blood.

Apoptosis

Our previous study[40] evaluated apoptotic cells after hUCBC administration to the HIE rat model. The number of caspase-3- (an apoptotic marker) positive cells in the hippocampus was significantly reduced in the UCBC group. Additionally, the number of apoptosis-inducing factor-positive cells, an initiator of caspase-independent apoptosis, was also reduced by the treatment. Rosenkranz *et al*.[33] showed that hUCBCs decreased HIE-induced apoptosis, judged by expression of cleaved caspase-3, whereas the number of neurons, identified by NeuN-positive cells, increased. Up-regulated brain-derived neurotrophic factor (BDNF) mRNA expression by UCBCs could contribute to the inhibition of apoptosis.

Other groups demonstrated similar hUCBC effects. Pimentel-Coelho *et al*.[22] showed that hUCBC transplantation reduced caspase-3-mediated cell death as well as degenerating neurons stained with Fluoro-Jade C in the striatum. Zhang *et al*.[47] and Grandvuillemin *et al*.[37] also reported the reduction of apoptotic cells (TUNEL-positive cells) in the ipsilateral hemisphere after UCBC treatment.

In Penny *et al*.'s evaluation[44] using multidose of UCBCs, caspase-3 positive cells in the somatosensory cortex and motor cortex were significantly decreased in the 3-dose group, but not in the 1-dose group.

Park *et al*.[42,43] showed that UCBC-derived MSC augmented the anti-apoptotic effect of hypothermia in the penumbra area.

Yu *et al*.[48] showed that either hUCB-CD34$^+$ cells or MNCs inhibit the expression of apoptosis-related genes (TNF-α, TNFR1, TNFR2, CD40, Fas) and decrease the activation of NF-κB in the HI-damaged brain. McDonald *et al*.[41] showed that hUCB-EPCs significantly attenuated the increased number of TUNEL-positive cells after HI.

These literatures show, therefore, that hUCBC treatment has anti-apoptotic properties against neonatal HI brain injury.

Oxidative stress

The suppression of oxidative stress after insult should be targeted in HIE,[17] with oxidative stress playing an important role in HI brain damage.[51] In our study[40] using hUCB-MNCs with a rat HIE model, oxidative stress markers, 4-hydroxy-2-nonenal (4HNE), and nitrotyrosine presented weaker expression in the hippocampus when UCBCs were administered 24 h after insult compared to the control.

Secretion of growth factors

Human UCB-MNC treatment up-regulates VEGF and BDNF mRNA expression in the brain.[33] Up-regulated VEGF may lead to enhanced angiogenesis and neurogenesis, and BDNF may lead to the exertion of anti-apoptotic effects or enhanced neuronal survival/neurogenesis. Yasuhara *et al*. also revealed elevated levels of glial cell derived neurotrophic factor, nerve growth factor, and BDNF 3 days after transplantation of hUCBC with enzyme-linked immunosorbent assay.[23]

Considering UCBCs secrete various important trophic factors, such as cytokines, angiogenic factors, and neurotrophic factors,[50] most of the positive treatment effects above may be exerted via these factors in a paracrine manner.

Conclusions

Umbilical cord blood cell treatment for HIE has various mechanisms, including paracrine effects, although the effect derived from engraftment and neuronal differentiation is limited. Most drugs, such as anti-inflammatory and anti-apoptotic drugs, which are expected to be new therapy candidates, need to be administered in the acute phase. In contrast, the administration of UCBCs, which have enhancing effects on endogenous neurogenesis and angiogenesis, can potentially exert a treatment effect in the subacute phase as well. In fact, several animal studies have reported a long therapeutic time window.[19,23] Considering the complex mechanism of injury in HIE, the diverse actions and wide therapeutic time windows of UCBC treatment are expected to exert more robust effects for a wider population of HIE babies than conventional therapies.

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Disclosure

The authors declare no conflict of interest.

Author contributions

YS and MT contributed to the conception and design of this review article; YS drafted the manuscript; MT critically reviewed the manuscript. Both authors read and approved the final manuscript.

References

1 Hug L, Alexander M, You D, Alkema L. National, regional, and global levels and trends in neonatal mortality between 1990 and 2017, with scenario-based projections to 2030: a systematic analysis. *Lancet Glob. Health.* 2019; **7**: e710–e20.
2 Touyama M, Touyama J, Toyokawa S, Kobayashi Y. Trends in the prevalence of cerebral palsy in children born between 1988 and 2007 in Okinawa, Japan. *Brain Dev.* 2016; **38**: 792–9.
3 Kurinczuk JJ, White-Koning M, Badawi N. Epidemiology of neonatal encephalopathy and hypoxic-ischaemic encephalopathy. *Early Human Dev.* 2010; **86**: 329–38.
4 Shankaran S, Laptook AR, Ehrenkranz RA *et al*. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N. Engl. J. Med.* 2005; **353**: 1574–84.
5 Gluckman PD, Wyatt JS, Azzopardi D *et al*. Selective head cooling with mild systemic hypothermia after neonatal

encephalopathy: multicentre randomised trial Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *Lancet.* 2005; **365**: 663–70.
6 Centers for Disease Control and Prevention. Economic costs associated with mental retardation. cerebral palsy, hearing loss, and vision impairment–United States, 2003. *MMWR Morb. Mortal Wkly. Rep.* 2004; **53**: 57–9.
7 Kruse M, Michelsen SI, Flachs EM, Brønnum-Hansen H, Madsen M, Uldall P. Lifetime costs of cerebral palsy. *Dev. Med. Child Neurol.* 2009; **51**: 622–28.
8 Edwards AD, Brocklehurst P, Gunn AJ *et al*. Neurological outcomes at 18 months of age after moderate hypothermia for perinatal hypoxic ischaemic encephalopathy: synthesis and meta-analysis of trial data. *BMJ* 2010; **340**: c363.
9 Lindvall O, Kokaia Z. Stem cells for the treatment of neurological disorders. *Nature* 2006; **441**: 1094–96.
10 Sato Y, Oohira A. Chondroitin sulfate, a major niche substance of neural stem cells, and cell transplantation therapy of neurodegeneration combined with niche modification. *Curr. Stem Cell. Res. Ther.* 2009; **4**: 200–9.
11 Sato Y, Nakanishi K, Hayakawa M *et al*. Reduction of brain injury in neonatal hypoxic-ischemic rats by intracerebroventricular injection of neural stem/progenitor cells together with chondroitinase ABC. *Reprod. Sci.* 2008; **15**: 613–20.
12 Sato Y, Shinjyo N, Sato M *et al*. Grafting of neural stem and progenitor cells to the hippocampus of young, irradiated mice causes gliosis and disrupts the granule cell layer. *Cell Death Dis.* 2013; **4**: e591.
13 Tsuji M. Hematopoietic stem cells for perinatal brain injury. In: Shintaku H, Oka A, Nabetani M (eds). *Cell Therapy for Perinatal Brain Injury*. Springer Singapore, Singapore, 2018; 45–56.
14 Tsuji M, Sawada M, Watabe S *et al*. Autologous cord blood cell therapy for neonatal hypoxic-ischaemic encephalopathy: a pilot study for feasibility and safety. *Sci. Rep.* 2020; **10**: 4603–03.
15 Cotten CM, Murtha AP, Goldberg RN *et al*. Feasibility of autologous cord blood cells for infants with hypoxic-ischemic encephalopathy. *J. Pediatr.* 2014; **164**: 973–79.e1.
16 Meirelles Lda S, Fontes AM, Covas DT, Caplan AI. Mechanisms involved in the therapeutic properties of mesenchymal stem cells. *Cytokine Growth Factor Rev.* 2009; **20**: 419–27.
17 Mikrogeorgiou A, Sato Y, Kondo T *et al.* Dedifferentiated fat cells as a novel source for cell therapy to target neonatal hypoxic-ischemic encephalopathy. *Dev. Neurosci.* 2017; **39**: 273–86.
18 Liao Y, Cotten M, Tan S, Kurtzberg J, Cairo MS. Rescuing the neonatal brain from hypoxic injury with autologous cord blood. *Bone Marrow Transplant.* 2013; **48**: 890–900.
19 Nakanishi K, Sato Y, Mizutani Y, Ito M, Hirakawa A, Higashi Y. Rat umbilical cord blood cells attenuate hypoxic-ischemic brain injury in neonatal rats. *Sci. Rep.* 2017; **7**: 44111.
20 Johnston MV, Fatemi A, Wilson MA, Northington F. Treatment advances in neonatal neuroprotection and neurointensive care. *Lancet. Neurol.* 2011; **10**: 372–82.
21 Meier C, Middelanis J, Wasielewski B *et al*. Spastic paresis after perinatal brain damage in rats is reduced by human cord blood mononuclear cells. *Pediatr. Res.* 2006; **59**: 244–9.
22 Pimentel-Coelho PM, Magalhaes ES, Lopes LM, deAzevedo LC, Santiago MF, Mendez-Otero R. Human cord blood transplantation in a neonatal rat model of hypoxic-ischemic brain damage: functional outcome related to neuroprotection in the striatum. *Stem Cells Dev.* 2010; **19**: 351–8.
23 Yasuhara T, Hara K, Maki M *et al*. Mannitol facilitates neurotrophic factor upregulation and behavioral recovery in neonatal hypoxic-ischemic rats with human umbilical cord blood grafts. *J. Cell. Mol. Med.* 2010; **14**: 914–21.
24 Bae SH, Kong TH, Lee HS *et al*. Long-lasting paracrine effects of human cord blood cells on damaged neocortex in an animal model of cerebral palsy. *Cell Transplant.* 2012; **21**: 2497–515.
25 Eriksson PS, Perfilieva E, Bjork-Eriksson T *et al*. Neurogenesis in the adult human hippocampus. *Nat. Med.* 1998; **4**: 1313–7.
26 Luskin MB. Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. *Neuron* 1993; **11**: 173–89.
27 Wang XL, Zhao YS, Hu MY, Sun YQ, Chen YX, Bi XH. Umbilical cord blood cells regulate endogenous neural stem cell proliferation via hedgehog signaling in hypoxic ischemic neonatal rats. *Brain Res.* 2013; **1518**: 26–35.
28 Wang X, Zhao Y, Wang X. Umbilical cord blood cells regulate the differentiation of endogenous neural stem cells in hypoxic ischemic neonatal rats via the hedgehog signaling pathway. *Brain Res.* 2014; **1560**: 18–26.
29 Baburamani AA, Castillo-Melendez M, Walker DW. VEGF expression and microvascular responses to severe transient hypoxia in the fetal sheep brain. *Pediatr. Res.* 2013; **73**: 310–6.
30 Li L, Xiong Y, Qu Y *et al*. The requirement of extracellular signal-related protein kinase pathway in the activation of hypoxia inducible factor 1α in the developing rat brain after hypoxia–ischemia. *Acta Neuropathol.* 2008; **115**: 297–303.
31 Feng Y, Rhodes PG, Bhatt AJ. Neuroprotective effects of vascular endothelial growth factor following hypoxic ischemic brain injury in neonatal rats. *Pediatr. Res.* 2008; **64**: 370–4.
32 Sun J, Sha B, Zhou W, Yang Y. VEGF-mediated angiogenesis stimulates neural stem cell proliferation and differentiation in the premature brain. *Biochem. Biophys. Res. Comm.* 2010; **394**: 146–52.
33 Rosenkranz K, Kumbruch S, Tenbusch M *et al*. Transplantation of human umbilical cord blood cells mediated beneficial effects on apoptosis, angiogenesis and neuronal survival after hypoxic-ischemic brain injury in rats. *Cell Tissue Res.* 2012; **348**: 429–38.
34 Cho KH, Choi JI, Kim JO, Jung JE, Kim DW, Kim M. Therapeutic mechanism of cord blood mononuclear cells via the IL-8-mediated angiogenic pathway in neonatal hypoxic-ischaemic brain injury. *Sci. Rep.* 2020; **10**: 4446.
35 Ohshima M, Taguchi A, Sato Y *et al*. Evaluations of intravenous administration of CD34+ human umbilical cord blood cells in a mouse model of neonatal hypoxic-ischemic encephalopathy. *Dev. Neurosci.* 2016; **38**: 331–41.
36 Tsuji M, Taguchi A, Ohshima M *et al*. Effects of intravenous administration of umbilical cord blood CD34 cells in a mouse model of neonatal stroke. *Neuroscience* 2014; **263C**: 148–58.
37 Grandvuillemin I, Garrigue P, Ramdani A *et al*. Long-term recovery after endothelial colony-forming cells or human umbilical cord blood cells administration in a rat model of neonatal hypoxic-ischemic encephalopathy. *Stem Cells Transl Med.* 2017; **6**: 1987–96.
38 Kitase Y, Sato Y, Ueda K *et al*. A novel treatment with stem cells from human exfoliated deciduous teeth for hypoxic-ischemic encephalopathy in neonatal rats. *Stem Cells Dev.* 2020; **29**: 63–74.
39 Sugiyama Y, Sato Y, Kitase Y *et al*. Intravenous administration of bone marrow-derived mesenchymal stem cell, but not adipose tissue-derived stem cell, ameliorated the neonatal hypoxic-ischemic brain injury by changing cerebral inflammatory state in rat. *Front. Neurol.* 2018; **9**: 757.
40 Hattori T, Sato Y, Kondo T *et al*. Administration of umbilical cord blood cells transiently decreased hypoxic-ischemic brain injury in neonatal rats. *Dev. Neurosci.* 2015; **37**: 95–104.

41 McDonald CA, Penny TR, Paton MCB *et al*. Effects of umbilical cord blood cells, and subtypes, to reduce neuroinflammation following perinatal hypoxic-ischemic brain injury. *J. Neuroinflammation* 2018; **15**: 47.
42 Park WS, Sung SI, Ahn SY *et al*. Hypothermia augments neuroprotective activity of mesenchymal stem cells for neonatal hypoxic-ischemic encephalopathy. *PLoS One* 2015; **10**: e0120893.
43 Ahn SY, Chang YS, Sung DK, Sung SI, Park WS. Hypothermia broadens the therapeutic time window of mesenchymal stem cell transplantation for severe neonatal hypoxic ischemic encephalopathy. *Sci. Rep.* 2018; **8**: 7665.
44 Penny TR, Pham Y, Sutherland AE *et al*. Multiple doses of umbilical cord blood cells improve long-term brain injury in the neonatal rat. *Brain Res.* 2020; **1746**: 147001.
45 Li B, Concepcion K, Meng X, Zhang L. Brain-immune interactions in perinatal hypoxic-ischemic brain injury. *Prog. Neurobiol.* 2017; **159**: 50–68.
46 Wasielewski B, Jensen A, Roth-Harer A, Dermietzel R, Meier C. Neuroglial activation and Cx43 expression are reduced upon transplantation of human umbilical cord blood cells after perinatal hypoxic-ischemic injury. *Brain Res.* 2012; **1487**: 39–53.
47 Zhang J, Yang C, Chen J *et al*. Umbilical cord mesenchymal stem cells and umbilical cord blood mononuclear cells improve neonatal rat memory after hypoxia-ischemia. *Behav. Brain Res.* 2019; **362**: 56–63.
48 Yu Y, Yan Y, Luo Z *et al*. Effects of human umbilical cord blood CD34(+) cell transplantation in neonatal hypoxic-ischemia rat model. *Brain Dev.* 2019; **41**: 173–81.
49 Saab AS, Tzvetanova ID, Nave KA. The role of myelin and oligodendrocytes in axonal energy metabolism. *Curr. Opin. Neurobiol.* 2013; **23**: 1065–72.
50 Baba N, Wang F, Iizuka M *et al*. Induction of regional chemokine expression in response to human umbilical cord blood cell infusion in the neonatal mouse ischemia-reperfusion brain injury model. *PLoS One* 2019; **14**: e0221111.
51 Warner DS. Oxidants, antioxidants and the ischemic brain. *J. Exp. Biol.* 2004; **207**: 3221–31.