Histol Histopathol (2023) 38: 965-974

Histology and Histopathology
From Cell Biology to Tissue Engineering

REVIEW Open Access

Development of a new treatment for preterm birth complications using amniotic fluid stem cell therapy

Yushi Abe[1], Yu Sato[1], Mamoru Tanaka[1] and Daigo Ochiai[1,2]

[1]Department of Obstetrics and Gynecology, Keio University School of Medicine, Tokyo and

[2]Department of Obstetrics and Gynecology, Kitasato University School of Medicine, Kanagawa, Japan

Summary. This paper describes the current status of studies and clinical trials on the use of mesenchymal stem cells (MSCs) and amniotic fluid stem cells (AFSCs) for complications of preterm birth (PTB), an urgent issue in the perinatal field. PTB is a serious challenge in clinical medicine that is increasing globally, and effective control of its complications is necessary for newborns' subsequent long life. Classical treatments are inadequate, and many patients have PTB complications. A growing body of evidence provided by translational medicine and others indicates that MSCs, and among them, the readily available AFSCs, may be useful in treating PTB complications. AFSCs are the only MSCs available prenatally and are known to be highly anti-inflammatory and tissue-protective and do not form tumors when transplanted. Furthermore, because they are derived from the amniotic fluid, a medical waste product, no ethical issues are involved. AFSCs are an ideal cell resource for MSC therapy in neonates. This paper targets the brain, lungs, and intestines, which are the vital organs most likely to be damaged by PTB complications. The evidence to date and future prospects with MSCs and AFSCs for these organs are described.

Key words: Amniotic fluid stem cell, Preterm birth

Introduction

Preterm birth (PTB) is an urgent and rising problem in perinatology. Even if preterm infants can be saved, PTB can damage vital organs and patients may have long-term sequelae. Reducing the sequelae is important for the quality of life of the patient's family and healthcare economics. There is currently no effective treatment for the unique complications of PTB, and evidence from *in vivo* and *in vitro* studies suggests that stem cell therapy for preterm complications may be an innovative treatment strategy. This article presents basic findings on the efficacy of amniotic fluid stem cell (AFSC) therapy for preterm complications arising in vital organs.

Stem cells are self-renewing and multipotent cells. Many types of stem cells have been described depending on their origin and the method used to create them. Growing evidence from numerous studies and clinical trials suggests that stem cell therapies have the potential to be innovative treatments for intractable diseases for which there are currently no effective therapies. AFSCs, which can be isolated from fetal amniotic fluid, are stem cells that can be isolated at the earliest stage of life. AFSCs are a promising source for stem cell therapies for fetuses and neonates (Fig. 1).

Amniotic fluid stem cells

AFSCs are a type of mesenchymal stem cell (MSC) and account for approximately 1% of the cells floating in amniotic fluid. These cells express CD117 (c-kit) and can be easily isolated by immunoselection (De Coppi et al., 2007). MSCs may be a potential cell source for regenerative medicine, which involves regenerating tissue lost due to injury. MSCs also have a potent anti-inflammatory effect and may be a cell source for cellular therapies that take advantage of this effect. Therefore, MSC therapy is expected to have tissue regenerative effects, anti-inflammatory effects, or both, and AFSCs have advantageous properties in both of these areas compared to other cells.

Ethics

Embryonic stem cells (ESCs), also known as representative stem cells, are inherently pluripotent. However, there are ethical concerns because creating ESCs requires the destruction of embryos (Lovell-Badge et al., 2021). In contrast, AFSCs can be isolated from amniotic fluid obtained during diagnostic amniocentesis

Corresponding Author: Daigo Ochiai, Department of Obstetrics and Gynecology, Kitasato University School of Medicine, 1-15-1, Kitasato, Sagamihara, Kanagawa 228-8555, Japan. e-mail: ochiaidaigo@gmail.com

www.hh.um.es. DOI: 10.14670/HH-18-607

and cesarean sections (Moraghebi et al., 2017). In the future, it may be possible to isolate AFSCs from amniotic fluid spilled during vaginal delivery or premature rupture of membrane (Hamid et al., 2017). Diagnostic amniocentesis is usually performed at 15-17 weeks of gestation. The miscarriage rate associated with this test is very low, and the procedure is well-established and safe (Salomon et al., 2019). The amniotic fluid that spills during a cesarean section or vaginal delivery is usually aspirated and disposed of as medical waste. Therefore, AFSCs have no additional patient burden or ethical issues.

Safety

Breakthroughs in regenerative medicine research have occurred with the development of induced pluripotent stem cells (iPSCs). iPSCs are obtained by transfecting somatic cells with four transcription factors (Takahashi et al., 2007). These reprogrammed cells are used in various preclinical studies involving regenerating damaged organs. However, iPSCs have been reported to form tumors *in vivo* (Miura et al., 2009) and accumulate genetic alterations upon repeated culture (Rouhani et al., 2022). In addition, the establishment of autologous iPSCs is costly and time-consuming, and allogeneic iPSCs can cause immune rejection. In contrast, AFSCs maintain genomic stability even after long-term culture and do not form tumors when administered to animals. When culturing AFSCs, no cumbersome procedures such as feeder cells are required. AFSCs adhere to plastic culture dishes and have a high proliferative capacity, allowing large numbers of cells to be obtained quickly. AFSCs are also positive for major histocompatibility complex (MHC) class I molecules and weakly positive for MHC class II molecules. These facts suggest that AFSCs are safe and suitable cells for clinical application (De Coppi et al., 2007; Davydova et al., 2009).

Therapeutic effect

Bone marrow (BM)- and adipose tissue (AT)-derived MSCs have been reported to have anti-inflammatory and anti-immunogenic properties in addition to their multipotent differentiation potential. These MSCs release many types of humoral factors, which act in an inhibitory manner on surrounding immune cells such as dendritic cells, monocytes, natural killer cells, T cells, and neutrophils (Aggarwal and Pittenger, 2005). In addition, MSCs themselves accumulate and migrate to sites of inflammation and tissue damage and exert tissue repair effects (Yagi et al., 2010). By utilizing the immunomodulatory and anti-inflammatory effects of MSCs, MSC therapy has been clinically applied to patients with graft-versus-host disease, Crohn disease, and knee osteoarthritis, who need to regulate excessive immune responses (Aggarwal and Pittenger, 2005; Levy et al., 2020). However, the development of MSC therapy for fetuses and neonates is less advanced than for adults. This is because autologous MSCs derived from BM-MSCs or AT-MSCs can only be isolated after birth and would require long-term monitoring of side effects and high safety requirements. AFSCs are a cell source that can solve these problems; AFSCs can be collected from amniocentesis at 15-17 weeks of gestation and are the earliest MSCs that can be isolated in a lifetime. As mentioned above, they are safe and have high anti-inflammatory activity comparable to other MSCs. Their efficacy has already been reported in animal models of diseases such as myelomeningocele (Abe et al., 2019), myocardial infarction, muscular dystrophy, acute tubular

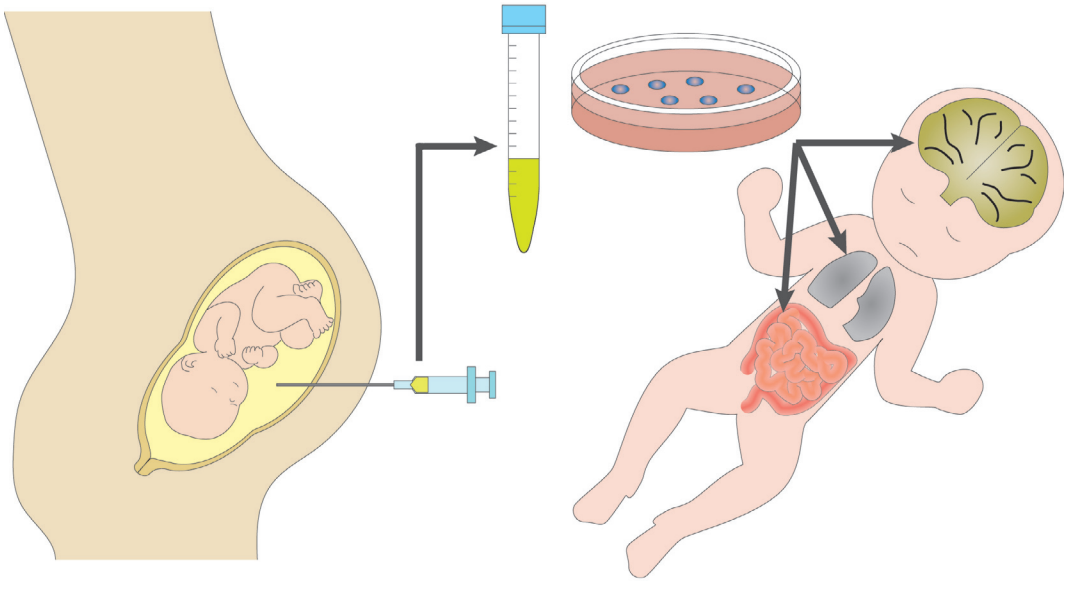

Fig. 1. Conceptual figure of autologous AFSC therapy for preterm complications. AFSCs are obtained from a small amount of amniotic fluid collected by amniocentesis during pregnancy, and expanded *in vitro*. The expanded AFSCs can then be used therapeutically for preterm complications immediately after delivery.

necrosis, lung injury (Cananzi and De Coppi, 2012), and stroke (Sibov et al., 2019). This paper focuses on the therapeutic effects of AFSCs on premature birth and its complications.

Preterm birth

PTB is defined as live birth occurring before 37 weeks of gestation. Owing to a lack of data from low-income countries, the true prevalence of PTB is unknown; estimates based on data from 107 countries indicate that approximately 15 million PTBs occur each year (Chawanpaiboon et al., 2019). Furthermore, annually, 1 million children worldwide die from PTB before the age of 5 years, accounting for an estimated 18% of all deaths in children younger than 5 years and 35% of all neonatal (younger than 28 days) deaths (Walani, 2020). Despite advances in neonatal intensive care, systemic inflammation due to neonatal sepsis in preterm infants is a major factor in preterm infant death (Shane et al., 2017). Preterm infants are known to develop various complications, including of the vital organs such as the brain, lungs, and intestines, which not only have a poor short-term prognosis but also require long-term treatment. Many survivors face lifelong disabilities such as learning disabilities and visual and hearing impairments that significantly impair their quality of life (Ward and Beachy, 2003). Currently, there are few effective treatments for these frequent complications of PTB; thus, there is an urgent need to develop new treatments based on innovative concepts.

Preterm birth complications

Brain damage

Background

Brain damage is one of the most fatal complications in preterm infants, and even in surviving infants, it often leads to long-term sequelae. Therefore, it is no exaggeration to say that controlling brain damage is the most important issue in improving the prognosis of preterm infants. Despite recent advances in neonatal care, preterm infants with underdeveloped brains are at higher risk of cerebral palsy (CP) caused by intraventricular hemorrhage (IVH), periventricular leukomalacia (PVL), and hypoxic-ischemic encephalopathy (HIE) than full-term infants (Linsell et al., 2016). In addition, because of the immaturity of the immune systems of preterm infants, the incidence of infectious diseases in preterm infants is high (Shane et al., 2017; Schuller et al., 2018), and when inflammation spreads throughout the body and sepsis occurs, brain damage may result. It has been reported that 4%-14% of the neurological sequelae among low-birth-weight infants are caused by neonatal sepsis (Haller et al., 2016). This is believed to be due to multiorgan failure caused by systemic inflammatory reactions, which leads to hypoperfusion and hypoxia in the brain and induces free radicals. This can irreversibly damage developing nerve cells and be a factor in poor long-term neurological prognosis (Hagberg and Mallard, 2005; Silveira et al., 2008; Comim et al., 2016; Barichello et al., 2019).

Brain damage and MSCs

Although there are various causes of brain damage in preterm infants, it is important to control excessive inflammation and inhibit damage to developing neurons in all cases. In recent years, animal experiments and clinical trials of MSC therapy for perinatal brain injury have been conducted. In the past, it was believed that transplanted stem cells proliferated in the damaged area, where they differentiated into neurons and replaced the destroyed tissue. However, the low proliferation and the insufficient rate of differentiation did not explain the significant neuronal improvement observed. Subsequent studies showed that MSCs transplanted into injured tissues release several factors, which induce the formation of neural stem cells, promote dendrite and axon expansion, and suppress inflammation after injury (Lee et al., 2010; van Velthoven et al., 2012).

Administration of human umbilical cord blood (UCB)-derived MSCs to a rat model of IVH has been shown to reduce post-IVH brain damage and post-hemorrhagic hydrocephalus (van Velthoven et al., 2012; Ahn et al., 2013, 2015; Park et al., 2016). Based on these findings, a phase I trial (NCT02274428) was conducted to evaluate the safety and feasibility of intracerebroventricular allogeneic transplantation of UCB-MSCs in premature infants with severe IVH. The results reported that the UCB-MSC transplantation treatment was safe (Ahn et al., 2018).

Recent studies of PVL have reported using a more clinically relevant double-hit model. This model recapitulates post-natal inflammation and hypoxia, resulting in transient deficits in cortical myelination and oligodendrocyte maturation, functional deficits, and neuroinflammation. Nasally administered BM-MSCs were shown to disperse and migrate into the injured brain, potently improving myelination and functional outcomes, suppressing brain inflammation, and rescuing oligodendrocyte maturation (Vaes et al., 2021).

HIE is a brain injury caused by hypoxia during delivery that occurs not only in preterm infants but also in full-term infants. In a mouse model study, nasal administration of BM-MSCs improved sensory-motor function and histological outcomes, a result maintained for at least 9 weeks after birth (Donega et al., 2013). Furthermore, intracerebroventricular administration of Wharton's jelly (WJ) MSCs resulted in protective activity characterized by decreased myelin loss and astroglial activation. Treatment with WJ-MSCs also provided relief from spastic paraplegia, restored motor symmetry, and improved gait patterns (Mueller et al., 2017).

The treatment mechanism of preterm brain injury by

MSCs is unclear. The therapeutic effect of administering only extracellular vesicles (EVs) secreted by MSCs (MSC-EVs) to an animal model of neonatal brain injury has been reported and was shown to significantly ameliorate inflammation-induced neuronal degeneration, reduce microgliosis, and prevent reactive astrogliosis (Ophelders et al., 2016; Drommelschmidt et al., 2017; Kaminski et al., 2020). However, a systematic study on AFSCs has not yet been reported. In the future, the therapeutic mechanism of MSC-EVs in different cell types should be investigated.

Brain damage and AFSCs

The therapeutic efficacy of MSC therapy for perinatal brain injury in humans has been reported to be limited (Cotten et al., 2014). There is no evidence from randomized trials evaluating the benefit or harm of stem cell therapy for HIE and IVH in neonates (Romantsik et al., 2019; Bruschettini et al., 2020). What are the reasons for the lack of therapeutic efficacy in humans, despite the high therapeutic efficacy reported so far in several animal models? We believe this is because the number of administered cells is insufficient. Collecting cord blood stem cells in preterm infants with low cord blood volume is difficult; therefore, the number of cells is insufficient when administered early after birth. Additionally, the administration of stem cells after culture is delayed because of the time required for culture.

AFSCs are the only source of stem cells that can be collected prenatally. By culturing the harvested cells, it is possible to obtain a large number of cells necessary for treatment before birth. The therapeutic effect of AFSC administration has been reported in a mouse model of HIE (Corcelli et al., 2018; Otani et al., 2019). In addition, the therapeutic effect on brain damage caused by neonatal sepsis has been shown in animal models (Abe et al., 2020). This study used a model of acute inflammation in which lipopolysaccharide (LPS) was administered at 3-4 days of age (P3-4). Previous preclinical reports have indicated that LPS administered at P3-4 causes acute inflammation involving reactive astrogliosis associated with microstructural alterations in the developing white matter (Nobuta et al., 2012). LPS administration induced inflammation in the whole body, including the brain, and intraperitoneal administration of AFSCs significantly reduced this inflammation (Sato et al., 2020). Furthermore, glial fibrillary acidic protein and ionized calcium-binding adapter protein 1, markers of gliosis and inflammation, were significantly reduced in the hippocampus of the AFSC-treated group, and the effects were maintained for 4 weeks. Significant improvements in higher brain functions, such as spatial cognition and short-term memory, were also observed (Abe et al., 2020). This was assessed by a behavioral test using the Barnes maze (McLay et al., 1998; Lin and Zuo, 2011). It is clear that cell numbers and dosing regimes are important for rescuing neuronal cells from hypoxia and low glucose-induced damage. When examining the treatment efficacy of various MSCs in the future, it is necessary to assess how many cells can actually be prepared immediately after birth.

Lung damage

Background

Bronchopulmonary dysplasia (BPD) is a common lung disorder in preterm infants (Gilfillan et al., 2021). BPD was reported by Northway et al. in 1967 as a respiratory disorder in preterm infants with characteristic chest radiographic and pathologic findings (Northway et al., 1967). The previous phenotype of BPD was fibrocystic disease seen in late preterm infants, but this is now uncommon. Today, most clinicians encounter BPD as a dysfunction of the alveoli and pulmonary vascular dysregulation, mainly in PTB before 29 weeks of gestation (Gilfillan et al., 2021). Currently, BPD is defined as a diagnosis in infants born at less than 32 weeks of gestation with radiographically confirmed persistent parenchymal lung lesions, and at 36 weeks post-menstrual age, requiring ventilator support for more than 3 days to maintain arterial oxygen saturation above 90%. As multiple factors contribute to the etiology of BPD, it has not been possible to adequately classify BPD. Chronic inflammation and hyperoxia are considered important mechanisms of BPD because they cause persistent airway and pulmonary vascular disease (Higgins et al., 2018). Despite advances in neonatal intensive care, BPD is a leading cause of mortality in both the short and long term. Disorders can persist beyond childhood and lead to lung disease in adults (Wong et al., 2008; Stoll et al., 2015).

BPD and MSCs

BM-MSCs and UCB-MSCs have been extensively studied in rodent models of BPD. MSC therapy for BPD can be divided into two categories, depending on the method of administration: intravenous and intratracheal.

When MSCs are administered intravenously, most are localized in the lungs (Eggenhofer et al., 2012). This means viable cells cannot pass through the lungs because MSCs are larger than pulmonary capillaries. Vasodilators have been used to reduce the first-passage effect (Schrepfer et al., 2007). In addition, several study reports showed that the cells observed in the lungs did not survive for more than 24 hours. However, longer persistency has been observed in later reports (Ferrini et al., 2021). Trapping by the lungs is a serious obstacle in MSC therapy for non-pulmonary organs, as it delays or reduces the delivery of the administered MSCs to the target organ. However, when targeting the lungs, this disadvantage becomes a strong advantage. Therefore, strategies to treat lung injury with cell-based therapies have been practiced in several animal models of lung disease. In particular, it has been studied for more than

20 years using animal models of radiation pneumonitis (Theise et al., 2002).

Multiple reports show MSCs are also effective against BPD (Augustine et al., 2017; Goetz et al., 2021). Notably, a study comparing the efficacy of BM-MSCs and their culture medium (CM) administered intravenously showed that the BM-MSC-CM had a higher therapeutic efficacy than BM-MSCs. Although systemically administered BM-MSCs demonstrated partial protection of lung structures, a single dose of BM-MSC-CM provided more complete protection and improved pulmonary vascular remodeling. These results clearly indicate that the therapeutic effect of intravenous MSC administration is not due to BM-MSCs differentiating into lung cells and improving alveolus structure but to secreted factors (secretomes) from the BM-MSCs (Aslam et al., 2009).

Intratracheal administration, conversely, can deliver larger amounts of cells more locally than systemic administration. Unlike the brain, the lung is one of the easier organs to administer to directly; intratracheal delivery of BM-MSCs to a BPD rat model improved the survival ratio and exercise tolerance and reduced alveolus and pulmonary vascular damage. Although some of the transplanted BM-MSCs were viable in lung tissue, the cell numbers were not sufficient to fully explain the therapeutic effect. This indicates that, as with intravenous delivery, the effects of the paracrine-mediated mechanism by BM-MSCs is important (van Haaften et al., 2009).

The world's first MSC product for BPD, for which there was no effective treatment, was PNEUMOSTEM®, produced by Medipost Co. Ltd (Gyeonggi, South Korea). This is a drug with MSCs derived from human UCB that is used for the treatment of BPD in preterm infants. A phase I clinical trial was initiated in 2010, demonstrating the safety and feasibility of the drug (NCT01297205), and subsequently, 2-year follow-up results were reported (NCT01632475). At a modified age of 2 years, there were no adverse effects on the respiratory system, growth, or neurodevelopment, or no adverse outcomes such as tumor formation (Ahn et al., 2017). A 5-year follow-up study on safety and efficacy in subjects who have completed phase II is currently underway (NCT04003857) and expected to be completed in 2027.

In addition to PNEUMOSTEM®, several other human clinical trials using stem cells for BPD exist. A clinical trial that currently recruiting is UMC119-01 (NCT03631420). The drug is an MSC derived from human umbilical cord stem cells, and it is manufactured by Meridigen Biotech Co. Ltd (Taipei, Taiwan). Many other clinical trials of MSC-based therapies for BPD have been designed and conducted, and most trials have reported no adverse events (Tang et al., 2022). The few adverse events that have occurred are presumed to be due to causes other than MSC transplantation. With the success of clinical trials, MSC preparations may become a promising option for BPD treatment.

BPD and AFSCs

AFSCs, which are autotransplantable and can have many cells prepared before delivery, may be more advantageous than UC-MSCs. Administration of AFSCs to a rat lung injury model after hyperoxic exposure showed significant therapeutic effects. AFSC administration increased the expression level of vascular endothelial growth factor (VEGF) in the lung, preserved the capillary structure of alveoli, and decreased the expression of inflammatory cytokines. In addition, immunohistochemistry of the AFSC-treated lungs was positive for both anti-human mitochondrial and anti-surfactant antibodies. Therefore, it was speculated that a portion of the administered AFSCs differentiated into alveolar type 2 cells (Grisafi et al., 2013). The potential for AFSCs to differentiate into type 2 alveolar epithelial cells has been shown in studies using animal models of chronic obstructive pulmonary disease (Li et al., 2014a,b; Lan et al., 2019) and *in vitro* (Li et al., 2014b).

The therapeutic effect of AFSCs with upregulated VEGF expression (AFSC-VEGF) on a rabbit lung injury model was greater than naive AFSCs. Treatment of a rabbit model of BPD with naive AFSCs reduced only lung inflammation and vascular structural defects. In contrast, treatment with AFSC-VEGF resulted in significant improvements in the structure and function of both lung parenchyma and blood vessels (Jiménez et al., 2018). These findings suggest that treatment with AFSCs for BPD may contribute to improving lung function by promoting alveolar formation and angiogenesis via VEGF. In summary, intratracheal or intravenous injection of AFSCs may be a promising new approach for the treatment of BPD. Future clinical trials of AFSC therapy should be based on the results of past MSC clinical trials to determine the optimal dose and method of administration.

Bowel damage

Background

Neonatal necrotizing enterocolitis (NEC) is a common complication of PTB. It is a life-threatening disease in preterm infants, with a mortality rate of approximately 15-30% (Niemarkt et al., 2019). Despite efforts to identify the disease's cause and develop a cure, the mortality rate remains high. Currently, the only effective treatment is surgical removal of the necrotic bowel. Patients who are treated surgically and survive may have severe sequelae. Long-term adverse events such as recurrence of NEC, narrowing of the intestinal tract, and neuro-developmental delay have been reported (Hintz et al., 2005; Neu and Walker, 2011; Heida et al., 2016). The microflora is believed to play an important role in the development of NEC, but the exact etiology

remains largely unknown (Niemarkt et al., 2019).

NEC and MSCs

In a rat model of NEC, intraperitoneal injection of BM-MSCs and homing MSCs to the site of injury reduced the incidence of NEC and improved clinical symptom scores (Tayman et al., 2011). This rat model was induced by twice-daily exposure to a +4°C cold environment for 5 min, exposure to 97% oxygen for 5 min, enteral feeding, and exposure to 100% carbon dioxide inhalation for 10 min (Guven et al., 2009). Using a similar rat model, BM-MSCs were also viable in the intestine after intravenous administration. Furthermore, when heparin-binding epidermal growth factor was used in combination, the therapeutic effect was greatly improved (Yang et al., 2012). Thus, considering multiple administration methods and their combination with growth factors greatly enhances the therapeutic effect of MSCs and helps to understand their therapeutic mechanism.

MSC-derived exosome-based therapies have also shown promising results. Treatment with exosomes has been reported to reduce the incidence and severity of disease as much as or more than MSCs. These are therefore another promising future therapeutic approach (Rager et al., 2016; McCulloh et al., 2018).

A case was reported in which MSC therapy was attempted for NEC. A 22-day-old boy delivered by cesarean section at 37 weeks of gestation had supraventricular tachycardia; paroxysmal ventricular tachycardia was treated with electrical cardioversion, but because of intestinal ischemia and revascularization, he developed NEC 14 hours later. After the necrotic bowel was resected by laparotomy, umbilical cord-derived MSCs were administered intravenously. As a result, the surviving intestine recovered, indicating that intravenous administration of MSCs may have contributed to the improvement (Akduman et al., 2021). This case report is encouraging for future NEC patients, but the efficacy of this treatment needs to be quantitatively examined in a clinical trial.

NEC and AFSCs

It is known that the nutritional factors in breast milk have a protective effect on newborns' intestines. Amniotic fluid contains the same growth factors as breast milk. Therefore, it is hypothesized that amniotic fluid has a protective effect against the development of NEC (Dasgupta and Jain, 2017). A study in a mouse model showed that enteral administration of amniotic fluid significantly reduced the extent of toll-like receptor 4 signaling in the intestinal mucosa (Good et al., 2012). A pig model study also showed a significant decrease in NEC score and secretion of pro-inflammatory cytokines (Siggers et al., 2013). This may be due to the involvement of nutritional factors (epidermal growth factor, hepatocyte growth factor, and transforming growth factor α) in amniotic fluid. Administration of nutritional factors alone has been shown to prevent the development of NEC (Dvorak et al., 2002; Maheshwari et al., 2011; Jain et al., 2014).

However, it has been hypothesized that AFSCs in amniotic fluid also contribute to the prevention of NEC. The first study reported the results of intraperitoneal administration of AFSCs in a mouse model of NEC. The administration of AFSCs significantly reduced the incidence and severity of NEC through a COX-2-dependent mechanism (Zani et al., 2014a). A subsequent study showed that AFSCs also prevented the development of ascites (Zani et al., 2014b).

In a study comparing the therapeutic effect on NEC by other stem cells, such as BM-MSCs and neonatal enteric neural stem cells, AFSCs showed a therapeutic effect comparable to other cells (McCulloh et al., 2017a,b). In a study using intestinal organoids, AFSCs reduced intestinal epithelial permeability and restored the localization of tight junctions. The development of NEC is associated with impaired intestinal barrier function. AFSC administration can reverse barrier function through activation of the endoplasmic reticulum stress response (Li et al., 2021).

Recently, exosomes have also been attracting attention as a fundamental part of the therapeutic mechanism of MSCs. Exosomes are granular substances with a diameter of 50-150 nm that are secreted by cells and contain nucleic acids and proteins. It has been reported that the nucleic acids in exosomes are transmitted to other cells and continue to function (Valadi et al., 2007). When exosomes derived from AFSCs were administered to a mouse model of NEC, they increased cell proliferation, reduced inflammation, and eventually regenerated normal intestinal epithelium. These therapeutic effects were attributed to exosomes activating the Wnt signaling pathway (Li et al., 2020). RNA-seq combined with modern bioinformatics analysis suggests that breast milk-derived exosomes and AFSC-derived exosomes may treat NEC by different mechanisms (Hu et al., 2022).

Conclusion

Complications in the vital organs (brain, lungs, and intestines) significantly impact the prognosis of preterm infants. AFSC therapy or AFSC-derived exosome therapy is a promising treatment for NEC. Although other stem cells have therapeutic effects, AFSCs are the only cell source that can be established during pregnancy and provide sufficient cells for preterm infants. Furthermore, they are as effective as or more effective than other MSCs. Multiple mechanisms may be involved in the therapeutic effects of AFSCs, and further research is needed to realize the translation to clinical application in humans. It is hoped that AFSC therapy will be successful in treating NEC, for which no effective treatment currently exists. AFSC treatment of complications of PTB may be a blessing for preterm infants.

Acknowledgements and funding sources. This work was supported by JSPS KAKENHI (grant numbers: JP19K22602 and JP21H03080) and JST SPRING (grant number: JPMJSP2123).
Conflicts of Interest. The authors declare no competing interests.

References

Abe Y., Ochiai D., Masuda H., Sato Y., Otani T., Fukutake M., Ikenoue S., Miyakoshi K., Okano H. and Tanaka M. (2019). *In utero* amniotic fluid stem cell therapy protects against myelomeningocele via spinal cord coverage and hepatocyte growth factor secretion. Stem Cells Transl. Med. 8, 1170-1179.

Abe Y., Ochiai D., Sato Y., Kanzaki S., Ikenoue S., Kasuga Y. and Tanaka M. (2020). Prophylactic therapy with human amniotic fluid stem cells improves long-term cognitive impairment in rat neonatal sepsis survivors. Int. J. Mol. Sci. 21, 9590.

Aggarwal S. and Pittenger M.F. (2005). Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 105, 1815-1822.

Ahn S.Y., Chang Y.S., Kim J.H., Sung S.I. and Park W.S. (2017). Two-year follow-up outcomes of premature infants enrolled in the phase I trial of mesenchymal stem cells transplantation for bronchopulmonary dysplasia. J. Pediatr. 185, 49-54. e2.

Ahn S.Y., Chang Y.S., Sung D.K., Sung S.I., Yoo H.S., Lee J.H., Oh W.I. and Park W.S. (2013). Mesenchymal stem cells prevent hydrocephalus after severe intraventricular hemorrhage. Stroke 44, 497-504.

Ahn S.Y., Chang Y.S., Sung D.K., Sung S.I., Yoo H.S., Im G.H., Choi S.J. and Park W.S. (2015). Optimal route for mesenchymal stem cells transplantation after severe intraventricular hemorrhage in newborn rats. PLoS One 10, e0132919.

Ahn S.Y., Chang Y.S., Sung S.I. and Park W.S. (2018). Mesenchymal stem cells for severe intraventricular hemorrhage in preterm infants: Phase I dose-escalation clinical trial. Stem Cells Transl. Med. 7, 847-856.

Akduman H., Dilli D., Ergün E., Çakmakçı E., Çelebi S.K., Çitli R. and Zenciroğlu A. (2021). Successful mesenchymal stem cell application in supraventricular tachycardia-related necrotizing enterocolitis: A case report. Fetal Pediatr. Pathol. 40, 250-255.

Aslam M., Baveja R., Liang O.D., Fernandez-Gonzalez A., Lee C., Mitsialis S.A. and Kourembanas S. (2009). Bone marrow stromal cells attenuate lung injury in a murine model of neonatal chronic lung disease. Am. J. Respir. Crit. Care Med. 180, 1122-1130.

Augustine S., Avey M.T., Harrison B., Locke T., Ghannad M., Moher D. and Thébaud B. (2017). Mesenchymal stromal cell therapy in bronchopulmonary dysplasia: Systematic review and meta-analysis of preclinical studies. Stem Cells Transl. Med. 6, 2079-2093.

Barichello T., Sayana P., Giridharan V.V., Arumanayagam A.S., Narendran B., Della Giustina A., Petronilho F., Quevedo J. and Dal-Pizzol F. (2019). Long-term cognitive outcomes after sepsis: A translational systematic review. Mol. Neurobiol. 56, 186-251.

Bruschettini M., Romantsik O., Moreira A., Ley D. and Thebaud B. (2020). Stem cell-based interventions for the prevention of morbidity and mortality following hypoxic-ischaemic encephalopathy in newborn infants. Cochrane Database Syst. Rev. 8, CD013202.

Cananzi M. and De Coppi P. (2012). CD117+ amniotic fluid stem cells: State of the art and future perspectives. Organogenesis 8, 77-88.

Chawanpaiboon S., Vogel J.P., Moller A.-B., Lumbiganon P., Petzold M., Hogan D., Landoulsi S., Jampathong N., Kongwattanakul K., Laopaiboon M., Lewis C., Rattanakanokchai S., Teng D.N., Thinkhamrop J., Watananirun K., Zhang J., Zhou W. and Gülmezoglu A.M. (2019). Global, regional, and national estimates of levels of preterm birth in 2014: A systematic review and modelling analysis. Lancet Global Health 7, e37-e46.

Comim C.M., Bussmann R.M., Simão S.R., Ventura L., Freiberger V., Patrício J.J., Palmas D., Mendonça B.P., Cassol O.J. Jr and Quevedo J. (2016). Experimental neonatal sepsis causes long-term cognitive impairment. Mol. Neurobiol. 53, 5928-5934.

Corcelli M., Hawkins K., Vlahova F., Hunjan A., Dowding K., De Coppi P., David A.L., Peebles D., Gressens P., Hagberg H., Hristova M. and Guillot P.V. (2018). Neuroprotection of the hypoxic-ischemic mouse brain by human CD117+CD90+CD105+ amniotic fluid stem cells. Sci. Rep. 8, 2425.

Cotten C.M., Murtha A.P., Goldberg R.N., Grotegut C.A., Smith P.B., Goldstein R.F., Fisher K.A., Gustafson K.E., Waters-Pick B., Swamy G.K., Rattray B., Tan S. and Kurtzberg J. (2014). Feasibility of autologous cord blood cells for infants with hypoxic-ischemic encephalopathy. J. Pediatr. 164, 973-979. e1.

Dasgupta S. and Jain S.K. (2017). Protective effects of amniotic fluid in the setting of necrotising enterocolitis. Pediatr. Res. 82, 584-595.

Davydova D.A., Vorotelyak E.A., Smirnova Y.A., Zinovieva R.D., Romanov Y.A., Kabaeva N.V., Terskikh V.V. and Vasiliev A.V. (2009). Cell phenotypes in human amniotic fluid. Acta Naturae 1, 98-103.

De Coppi P., Bartsch G., Siddiqui M.M., Xu T., Santos C.C., Perin L., Mostoslavsky G., Serre A.C., Snyder E.Y., Yoo J.J., Furth M.E., Soker S. and Atala A. (2007). Isolation of amniotic stem cell lines with potential for therapy. Nat. Biotechnol. 25, 100-106.

Donega V., van Velthoven C.T., Nijboer C.H., van Bel F., Kas M.J., Kavelaars A. and Heijnen C.J. (2013). Intranasal mesenchymal stem cell treatment for neonatal brain damage: Long-term cognitive and sensorimotor improvement. PLoS One 8, e51253.

Drommelschmidt K., Serdar M., Bendix I., Herz J., Bertling F., Prager S., Keller M., Ludwig A.K., Duhan V., Radtke S., de Miroschedji K., Horn P.A., van de Looij Y., Giebel B. and Felderhoff-Muser U. (2017). Mesenchymal stem cell-derived extracellular vesicles ameliorate inflammation-induced preterm brain injury. Brain Behav. Immun. 60, 220-232.

Dvorak B., Halpern M.D., Holubec H., Williams C.S., McWilliam D.L., Dominguez J.A., Stepankova R., Payne C.M. and McCuskey R.S. (2002). Epidermal growth factor reduces the development of necrotising enterocolitis in a neonatal rat model. Am. J. Physiol. Gastrointest. Liver Physiol. 282, G156-G164.

Eggenhofer E., Benseler V., Kroemer A., Popp F., Geissler E., Schlitt H., Baan C., Dahlke M. and Hoogduijn M. (2012). Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. Front. Immunol. 3, 297.

Ferrini E., Stellari F.F., Franceschi V., Macchi F., Russo L., Murgia A., Grisendi G., Villetti G., Dominici M. and Donofrio G. (2021). Persistency of mesenchymal stromal/stem cells in lungs. Front. Cell. Dev. Biol. 9, 709225.

Gilfillan M., Bhandari A. and Bhandari V. (2021). Diagnosis and management of bronchopulmonary dysplasia. BMJ 375, n1974.

Goetz M.J., Kremer S., Behnke J., Staude B., Shahzad T., Holzfurtner L., Chao C.-M., Morty R.E., Bellusci S. and Ehrhardt H. (2021). MSC based therapies to prevent or treat BPD-A narrative review on advances and ongoing challenges. Int. J. Mol. Sci. 22, 1138.

Good M., Siggers R.H., Sodhi C.P., Afrazi A., Alkhudari F., Egan C.E., Neal M.D., Yazji I., Jia H., Lin J., Branca M.F., Ma C., Prindle T., Grant Z., Shah S., Slagle D., 2nd Paredes J., Ozolek J., Gittes G.K. and Hackam D.J. (2012). Amniotic fluid inhibits Toll-like receptor 4 signaling in the fetal and neonatal intestinal epithelium. Proc. Natl. Acad. Sci. USA 109, 11330-11335.

Grisafi D., Pozzobon M., Dedja A., Vanzo V., Tomanin R., Porzionato A., Macchi V., Salmaso R., Scarpa M., Cozzi E., Fassina A., Navaglia F., Maran C., Onisto M., Caenazzo L., De Coppi P., De Caro R., Chiandetti L. and Zaramella P. (2013). Human amniotic fluid stem cells protect rat lungs exposed to moderate hyperoxia. Pediatr. Pulmonol. 48, 1070-1080.

Guven A., Gundogdu G., Vurucu S., Uysal B., Oztas E., Ozturk H. and Korkmaz A. (2009). Medical ozone therapy reduces oxidative stress and intestinal damage in an experimental model of necrotising enterocolitis in neonatal rats. J. Pediatr. Surg. 44, 1730-1735.

Hagberg H. and Mallard C. (2005). Effect of inflammation on central nervous system development and vulnerability. Curr. Opin. Neurol. 18, 117-123.

Haller S., Deindl P., Cassini A., Suetens C., Zingg W., Abu Sin M., Velasco E., Weiss B., Ducomble T., Sixtensson M., Eckmanns T. and Harder T. (2016). Neurological sequelae of healthcare-associated sepsis in very-low-birthweight infants: Umbrella review and evidence-based outcome tree. Euro Surveill. 21, 30143.

Hamid A.A., Joharry M.K., Mun-Fun H., Hamzah S.N., Rejali Z., Yazid M.N., Thilakavathy K. and Nordin N. (2017). Highly potent stem cells from full-term amniotic fluid: A realistic perspective. Reprod. Biol. 17, 9-18.

Heida F.H., Loos M.H., Stolwijk L., Te Kiefte B.J., van den Ende S.J., Onland W., van Rijn R.R., Dikkers R., van den Dungen F.A., Kooi E.M., Bos A.F., Hulscher J.B. and Bakx R. (2016). Risk factors associated with postnecrotizing enterocolitis strictures in infants. J. Pediatr. Surg. 51, 1126-1130.

Higgins R.D., Jobe A.H., Koso-Thomas M., Bancalari E., Viscardi R.M., Hartert T.V., Ryan R.M., Kallapur S.G., Steinhorn R.H., Konduri G.G., Davis S.D., Thebaud B., Clyman R.I., Collaco J.M., Martin C.R., Woods J.C., Finer N.N. and Raju T.N.K. (2018). Bronchopulmonary dysplasia: Executive summary of a workshop. J. Pediatr. 197, 300-308.

Hintz S.R., Kendrick D.E., Stoll B.J., Vohr B.R., Fanaroff A.A., Donovan E.F., Poole W.K., Blakely M.L., Wright L., Higgins R. and Network N.N.R. (2005). Neurodevelopmental and growth outcomes of extremely low birth weight infants after necrotising enterocolitis. Pediatrics. 115, 696-703.

Hu X., Zhang R., Liang H., An J., Yang Y., Huo J., Chen Z., Quan W., Jiang L., Li C., Li J., Li F., Xu Y. and Zhu X. (2022). Comparison and investigation of exosomes from human amniotic fluid stem cells and human breast milk in alleviating neonatal necrotizing enterocolitis. Stem Cell Rev. Rep. 19, 754-766.

Jain S.K., Baggerman E.W., Mohankumar K., Namachivayam K., Jagadeeswaran R., Reyes V.E. and Maheshwari A. (2014). Amniotic fluid-borne hepatocyte growth factor protects rat pups against experimental necrotising enterocolitis. Am. J. Physiol. Gastrointest. Liver Physiol. 306, G361-G369.

Jiménez J., Lesage F., Richter J., Nagatomo T., Salaets T., Zia S., Mori Da Cunha M.G., Vanoirbeek J., Deprest J.A. and Toelen J. (2018). Upregulation of vascular endothelial growth factor in amniotic fluid stem cells enhances their potential to attenuate lung injury in a preterm rabbit model of bronchopulmonary dysplasia. Neonatology 113, 275-285.

Kaminski N., Koster C., Mouloud Y., Borger V., Felderhoff-Muser U., Bendix I., Giebel B. and Herz J. (2020). Mesenchymal stromal cell-derived extracellular vesicles reduce neuroinflammation, promote neural cell proliferation and improve oligodendrocyte maturation in neonatal hypoxic-ischemic brain injury. Front. Cell. Neurosci. 14, 601176.

Lan Y.W., Yang J.C., Yen C.C., Huang T.T., Chen Y.C., Chen H.L., Chong K.Y. and Chen C.M. (2019). Predifferentiated amniotic fluid mesenchymal stem cells enhance lung alveolar epithelium regeneration and reverse elastase-induced pulmonary emphysema. Stem Cell Res. Ther. 10, 163.

Lee J.A., Kim B.I., Jo C.H., Choi C.W., Kim E.K., Kim H.S., Yoon K.S. and Choi J.H. (2010). Mesenchymal stem-cell transplantation for hypoxic-ischemic brain injury in neonatal rat model. Pediatr. Res. 67, 42-46.

Levy O., Kuai R., Siren E.M.J., Bhere D., Milton Y., Nissar N., De Biasio M., Heinelt M., Reeve B., Abdi R., Alturki M., Fallatah M., Almalik A., Alhasan A.H., Shah K. and Karp J.M. (2020). Shattering barriers toward clinically meaningful MSC therapies. Sci. Adv. 6, eaba6884.

Li Y., Gu C., Xu W., Yan J., Xia Y., Ma Y., Chen C., He X. and Tao H. (2014a). Therapeutic effects of amniotic fluid-derived mesenchymal stromal cells on lung injury in rats with emphysema. Respir. Res. 15, 120.

Li Y., Xu W., Yan J., Xia Y., Gu C., Ma Y. and Tao H. (2014b). Differentiation of human amniotic fluid-derived mesenchymal stem cells into type II alveolar epithelial cells *in vitro*. Int. J. Mol. Med. 33, 1507-1513.

Li B., Lee C., O'Connell J.S., Antounians L., Ganji N., Alganabi M., Cadete M., Nascimben F., Koike Y., Hock A., Botts S.R., Wu R.Y., Miyake H., Minich A., Maalouf M.F., Zani-Ruttenstock E., Chen Y., Johnson-Henry K.C., De Coppi P., Eaton S., Maattanen P., Olguin P.D., Zani A., Sherman P.M. and Pierro A. (2020). Activation of Wnt signaling by amniotic fluid stem cell-derived extracellular vesicles attenuates intestinal injury in experimental necrotising enterocolitis. Cell Death Dis. 11, 750.

Li B., Lee C., Chuslip S., Lee D., Biouss G., Wu R., Koike Y., Miyake H., Ip W., Gonska T. and Pierro A. (2021). Intestinal epithelial tight junctions and permeability can be rescued through the regulation of endoplasmic reticulum stress by amniotic fluid stem cells during necrotising enterocolitis. FASEB J. 35, e21265.

Lin D. and Zuo Z. (2011). Isoflurane induces hippocampal cell injury and cognitive impairments in adult rats. Neuropharmacology 61, 1354-1359.

Linsell L., Malouf R., Morris J., Kurinczuk J.J. and Marlow N. (2016). Prognostic factors for cerebral palsy and motor impairment in children born very preterm or very low birthweight: A systematic review. Dev. Med. Child. Neurol. 58, 554-569.

Lovell-Badge R., Anthony E., Barker R.A., Bubela T., Brivanlou A.H., Carpenter M., Charo R.A., Clark A., Clayton E., Cong Y., Daley G.Q., Fu J., Fujita M., Greenfield A., Goldman S.A., Hill L., Hyun I., Isasi R., Kahn J., Kato K., Kim J., Kimmelman J., Knoblich J.A., Mathews D., Montserrat N., Mosher J., Munsie M., Nakauchi H., Naldini L., Naughton G., Niakan K., Ogbogu U., Pedersen R., Rivron N., Rooke H., Rossant J., Round J., Saitou M., Sipp D., Steffann J., Sugarman J., Surani A., Takahashi J., Tang F., Turner L., Zettler P.J., Zhai X. (2021). ISSCR guidelines for stem cell research and clinical translation: The 2021 update. Stem Cell Rep. 16, 1398-1408.

Maheshwari A., Kelly D.R., Nicola T., Ambalavanan N., Jain S.K., Murphy-Ullrich J., Athar M., Shimamura M., Bhandari V., Aprahamian C., Dimmitt R.A., Serra R. and Ohls R.K. (2011). TGF-β2 suppresses macrophage cytokine production and mucosal inflammatory responses in the developing intestine. Gastroenterology 140, 242-253.

McCulloh C.J., Olson J.K., Wang Y., Vu J., Gartner S. and Besner G.E. (2017a). Evaluating the efficacy of different types of stem cells in preserving gut barrier function in necrotising enterocolitis. J. Surg. Res. 214, 278-285.

McCulloh C.J., Olson J.K., Zhou Y., Wang Y. and Besner G.E. (2017b). Stem cells and necrotising enterocolitis: A direct comparison of the efficacy of multiple types of stem cells. J. Pediatr. Surg. 52, 999-1005.

McCulloh C.J., Olson J.K., Wang Y., Zhou Y., Tengberg N.H., Deshpande S. and Besner G.E. (2018). Treatment of experimental necrotising enterocolitis with stem cell-derived exosomes. J. Pediatr. Surg. 53, 1215-1220.

McLay R.N., Freeman S.M. and Zadina J.E. (1998). Chronic corticosterone impairs memory performance in the Barnes maze. Physiol. Behav. 63, 933-937.

Miura K., Okada Y., Aoi T., Okada A., Takahashi K., Okita K., Nakagawa M., Koyanagi M., Tanabe K., Ohnuki M., Ogawa D., Ikeda E., Okano H. and Yamanaka S. (2009). Variation in the safety of induced pluripotent stem cell lines. Nat. Biotechnol. 27, 743-745.

Moraghebi R., Kirkeby A., Chaves P., Rönn R.E., Sitnicka E., Parmar M., Larsson M., Herbst A. and Woods N.-B. (2017). Term amniotic fluid: An unexploited reserve of mesenchymal stromal cells for reprogramming and potential cell therapy applications. Stem Cell Res. Ther. 8, 190.

Mueller M., Oppliger B., Joerger-Messerli M., Reinhart U., Barnea E., Paidas M., Kramer B.W., Surbek D.V. and Schoeberlein A. (2017). Wharton's jelly mesenchymal stem cells protect the immature brain in rats and modulate cell fate. Stem Cells Dev. 26, 239-248.

Neu J. and Walker W.A. (2011). Necrotizing enterocolitis. N. Engl. J. Med. 364, 255-264.

Niemarkt H.J., De Meij T.G., van Ganzewinkel C., de Boer N.K.H., Andriessen P., Hütten M.C. and Kramer B.W. (2019). Necrotizing enterocolitis, gut microbiota, and brain development: Role of the brain-gut axis. Neonatology 115, 423-431.

Nobuta H., Ghiani C.A., Paez P.M., Spreuer V., Dong H., Korsak R.A., Manukyan A., Li J., Vinters H.V., Huang E.J., Rowitch D.H., Sofroniew M.V., Campagnoni A.T., de Vellis J. and Waschek J.A. (2012). STAT3-mediated astrogliosis protects myelin development in neonatal brain injury. Ann. Neurol. 72, 750-765.

Northway W.H. Jr, Rosan R.C. and Porter D.Y. (1967). Pulmonary disease following respirator therapy of hyaline-membrane disease. Bronchopulmonary dysplasia. N. Engl. J. Med. 276, 357-368.

Ophelders D.R., Wolfs T.G., Jellema R.K., Zwanenburg A., Andriessen P., Delhaas T., Ludwig A.K., Radtke S., Peters V., Janssen L., Giebel B. and Kramer B.W. (2016). Mesenchymal stromal cell-derived extracellular vesicles protect the fetal brain after hypoxia-ischemia. Stem Cells Transl. Med. 5, 754-763.

Otani T., Ochiai D., Masuda H., Abe Y., Fukutake M., Matsumoto T., Miyakoshi K. and Tanaka M. (2019). The neurorestorative effect of human amniotic fluid stem cells on the chronic phase of neonatal hypoxic-ischemic encephalopathy in mice. Pediatr. Res. 85, 97-104.

Park W.S., Sung S.I., Ahn S.Y., Sung D.K., Im G.H., Yoo H.S., Choi S.J. and Chang Y.S. (2016). Optimal timing of mesenchymal stem cell therapy for neonatal intraventricular hemorrhage. Cell Transplant. 25, 1131-1144.

Rager T.M., Olson J.K., Zhou Y., Wang Y. and Besner G.E. (2016). Exosomes secreted from bone marrow-derived mesenchymal stem cells protect the intestines from experimental necrotising enterocolitis. J. Pediatr. Surg. 51, 942-947.

Romantsik O., Bruschettini M., Moreira A., Thebaud B. and Ley D. (2019). Stem cell-based interventions for the prevention and treatment of germinal matrix-intraventricular haemorrhage in preterm infants. Cochrane Database Syst. Rev. 9, CD013201.

Rouhani F.J., Zou X., Danecek P., Badja C., Amarante T.D., Koh G., Wu Q., Memari Y., Durbin R., Martincorena I., Bassett A.R., Gaffney D. and Nik-Zainal S. (2022). Substantial somatic genomic variation and selection for BCOR mutations in human induced pluripotent stem cells. Nat. Genet. 54, 1406-1416.

Salomon L.J., Sotiriadis A., Wulff C.B., Odibo A. and Akolekar R. (2019). Risk of miscarriage following amniocentesis or chorionic villus sampling: Systematic review of literature and updated meta-analysis. Ultrasound Obstet. Gynecol. 54, 442-451.

Sato Y., Ochiai D., Abe Y., Masuda H., Fukutake M., Ikenoue S., Kasuga Y., Shimoda M., Kanai Y. and Tanaka M. (2020). Prophylactic therapy with human amniotic fluid stem cells improved survival in a rat model of lipopolysaccharide-induced neonatal sepsis through immunomodulation via aggregates with peritoneal macrophages. Stem Cell Res. Ther. 11, 300.

Schrepfer S., Deuse T., Reichenspurner H., Fischbein M.P., Robbins R.C. and Pelletier M.P. (2007). Stem cell transplantation: The lung barrier. Transplant. Proc. 39, 573-576.

Schuller S.S., Kramer B.W., Villamor E., Spittler A., Berger A. and Levy O. (2018). Immunomodulation to prevent or treat neonatal sepsis: Past, present, and future. Front. Pediatr. 6, 199.

Shane A.L., Sánchez P.J. and Stoll B.J. (2017). Neonatal sepsis. Lancet 390, 1770-1780.

Sibov T.T., Pavon L.F., Cabral F.R., Cunha I.F., de Oliveira D.M., de Souza J.G., Marti L.C., da Cruz E.F., Malheiros J.M., Paiva F.F., Tannús A., de Oliveira S.M., da Costa M.D.S., Dastoli P.A., Mendonça J.N., de Toledo S.R.C., Malheiros S.M.F., de Paiva Neto M.A., Rego N.B.B., Moron A.F. and Cavalheiro S. (2019). Intravenous grafts of human amniotic fluid-derived stem cells reduce behavioral deficits in experimental ischemic stroke. Cell Transplant. 28, 1306-1320.

Siggers J., Ostergaard M.V., Siggers R.H., Skovgaard K., Molbak L., Thymann T., Schmidt M., Moller H.K., Purup S., Fink L.N., Frokiaer H., Boye M., Sangild P.T. and Bering S.B. (2013). Postnatal amniotic fluid intake reduces gut inflammatory responses and necrotising enterocolitis in preterm neonates. Am. J. Physiol. Gastrointest. Liver Physiol. 304, G864-G875.

Silveira R.C., Procianoy R.S., Dill J.C. and da Costa C.S. (2008). Periventricular leukomalacia in very low birth weight preterm neonates with high risk for neonatal sepsis. J. Pediatr. (Rio J.) 84, 211-216.

Stoll B.J., Hansen N.I., Bell E.F., Walsh M.C., Carlo W.A., Shankaran S., Laptook A.R., Sánchez P.J., Van Meurs K.P., Wyckoff M., Das A., Hale E.C., Ball M.B., Newman N.S., Schibler K., Poindexter B.B., Kennedy K.A., Cotten C.M., Watterberg K.L., D'Angio C.T., DeMauro S.B., Truog W.E., Devaskar U., Higgins R.D. andEunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network (2015). Trends in care practices, morbidity, and mortality of extremely preterm neonates,

1993-2012. JAMA 314, 1039-1051.

Takahashi K., Tanabe K., Ohnuki M., Narita M., Ichisaka T., Tomoda K. and Yamanaka S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131, 861-872.

Tang E., Zaidi M., Lim W.-H., Govindasamy V., Then K.-Y., Then K.-L., Das A.K. and Cheong S.-K. (2022). Headway and the remaining hurdles of mesenchymal stem cells therapy for bronchopulmonary dysplasia. Clin. Respir. J. 16, 629-645.

Tayman C., Uckan D., Kilic E., Ulus A.T., Tonbul A., Murat Hirfanoglu I., Helvacioglu F., Haltas H., Koseoglu B. and Tatli M.M. (2011). Mesenchymal stem cell therapy in necrotizing enterocolitis: A rat study. Pediatr. Res. 70, 489-494.

Theise N.D., Henegariu O., Grove J., Jagirdar J., Kao P.N., Crawford J.M., Badve S., Saxena R. and Krause D.S. (2002). Radiation pneumonitis in mice: A severe injury model for pneumocyte engraftment from bone marrow. Exp. Hematol. 30, 1333-1338.

Vaes J.E.G., van Kammen C.M., Trayford C., van der Toorn A., Ruhwedel T., Benders M., Dijkhuizen R.M., Mobius W., van Rijt S.H. and Nijboer C.H. (2021). Intranasal mesenchymal stem cell therapy to boost myelination after encephalopathy of prematurity. Glia 69, 655-680.

Valadi H., Ekstrom K., Bossios A., Sjostrand M., Lee J.J. and Lotvall J.O. (2007). Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat. Cell. Biol. 9, 654-659.

van Haaften T., Byrne R., Bonnet S., Rochefort G.Y., Akabutu J., Bouchentouf M., Rey-Parra G.J., Galipeau J., Haromy A., Eaton F., Chen M., Hashimoto K., Abley D., Korbutt G., Archer S.L. and Thébaud B. (2009). Airway delivery of mesenchymal stem cells prevents arrested alveolar growth in neonatal lung injury in rats. Am. J. Respir. Crit. Care Med. 180, 1131-1142.

van Velthoven C.T., Kavelaars A. and Heijnen C.J. (2012). Mesenchymal stem cells as a treatment for neonatal ischemic brain damage. Pediatr. Res. 71, 474-481.

Walani S.R. (2020). Global burden of preterm birth. Int. J. Gynaecol. Obstet. 150, 31-33.

Ward R.M. and Beachy J.C. (2003). Neonatal complications following preterm birth. BJOG 110, 8-16.

Wong P.M., Lees A.N., Louw J., Lee F.Y., French N., Gain K., Murray C.P., Wilson A. and Chambers D.C. (2008). Emphysema in young adult survivors of moderate-to-severe bronchopulmonary dysplasia. Eur. Respir. J. 32, 321-328.

Yagi H., Soto-Gutierrez A., Parekkadan B., Kitagawa Y., Tompkins R.G., Kobayashi N. and Yarmush M.L. (2010). Mesenchymal stem cells: Mechanisms of immunomodulation and homing. Cell Transplant. 19, 667-679.

Yang J., Watkins D., Chen C.-L., Bhushan B., Zhou Y. and Besner G.E. (2012). Heparin-binding epidermal growth factor-like growth factor and mesenchymal stem cells act synergistically to prevent experimental necrotizing enterocolitis. J. Am. Coll. Surg. 215, 534-545.

Zani A., Cananzi M., Fascetti-Leon F., Lauriti G., Smith V.V., Bollini S., Ghionzoli M., D'Arrigo A., Pozzobon M., Piccoli M., Hicks A., Wells J., Siow B., Sebire N.J., Bishop C., Leon A., Atala A., Lythgoe M.F., Pierro A., Eaton S., De Coppi P. (2014a). Amniotic fluid stem cells improve survival and enhance repair of damaged intestine in necrotising enterocolitis via a COX-2 dependent mechanism. Gut 63, 300-309.

Zani A., Cananzi M., Lauriti G., Fascetti-Leon F., Wells J., Siow B., Lythgoe M.F., Pierro A., Eaton S. and De Coppi P. (2014b). Amniotic fluid stem cells prevent development of ascites in a neonatal rat model of necrotising enterocolitis. Eur. J. Pediatr. Surg. 24, 57-60.

Accepted March 15, 2023