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# Changes in growth factor levels in the cerebrospinal fluid of autism patients after transplantation of human umbilical cord blood mononuclear cells and umbilical cord-derived mesenchymal stem cells

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**ABSTRACT.** The aim of the current study was to evaluate the levels of growth factors in the cerebrospinal fluid (CSF) of patients with autism, after transplantation of human umbilical cord blood mononuclear cells (CBMNCs) and umbilical cord-derived mesenchymal stem cells (UCMSCs). Twenty patients received two CBMNC intravenous and intrathecal infusions, each followed by two UCMSC intrathecal injections. A 2-mL sample of CSF was taken before each intrathecal injection. CSF levels of hepatocyte growth factor (HGF), brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) and basic fibroblast growth factor (bFGF) were determined by an enzyme-linked immunosorbent assay (ELISA). All data are reported as means  $\pm$  SD

and were analyzed using the SPSS 10.0 software. One-way analysis of variance with post-hoc F- and Q-tests was performed for comparison. HGF, BDNF and NGF levels in the CSF were significantly increased after transplantation ( $P < 0.05$ ), while bFGF levels did not change significantly. Therefore, transplantation of CBMNCs and UCMSCs could increase HGF, BDNF and NGF levels in the CSF of patients with autism.

**Key words:** Autism; Umbilical cord blood mononuclear cell; Umbilical cord-derived mesenchymal stem cell; Growth factors

## INTRODUCTION

Stem cells are highly proliferative, self-renewing, multi-potent cells with the potential to treat human degenerative diseases. In recent years, stem cell therapy has shown promise in the treatment of a number of human diseases. For example, stem cells from the bone marrow and umbilical cord blood have been used to treat leukemia (Park and Lee, 2013). Umbilical cord-derived mesenchymal stem cells (UCMSCs) from humans are abundant, self-renewing multi-potent stem cells with a differentiation potential towards neural lineage, and the ability to secrete growth and neurotrophic factors (Neuss et al., 2004; Yuan and Barker, 2005). As one potential source of neural stem cells, CBMNCs contain various progenitor cells including a large population of CD34<sup>+</sup> hematopoietic stem cells, a small population of mesenchymal stem cells, and very few endothelial progenitor cells and muscle satellite cells. These progenitor cells are highly proliferative with the potential to differentiate towards a neural cell fate, and secrete various growth and neurotrophic factors (Chen et al., 2005; Fan et al., 2005; Habich et al., 2006; Chen et al., 2007; Lee et al., 2007; Pachatz et al., 2008).

Transplantation of umbilical cord blood mononuclear cells (CBMNCs) and umbilical cord-derived mesenchymal stem cells (UCMSCs) showed therapeutic efficacy in patients with autism based on the index of Childhood Autism Rating Scale (CARS), Clinical Global Impression (CGI) scale, and Aberrant Behavior Checklist (ABC) (Lv et al., 2013). However, the underlying mechanism remains elusive. We recently reported that transplantation of CBMNCs increases levels of nerve growth factor in the cerebrospinal fluid of patients with autism (Li et al., 2013). In the current study, we investigated changes in the levels of hepatocyte growth factor (HGF), brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) and basic fibroblast growth factor (bFGF) in the cerebrospinal fluid (CSF) of autism patients after transplantation of CBMNCs and UCMSCs.

## MATERIAL AND METHODS

### Study subjects

The study protocol and consent forms were approved by the Institutional Review Board of Shandong Jiaotong Hospital under the auspices of the National Ministry of Health. Subjects were recruited from Shandong Jiaotong Hospital between March 2009 and August 2010. Eligible subjects in this study included 16 boys and 4 girls (3-14 years old) diagnosed with autism, in accordance with the diagnostic criteria for autism in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (Filipek et al., 1999; Mitchell et al.,

2006), and with a Childhood Autism Rating Scale (CARS) score  $\geq 30$ . The study protocols were approved by the Ethics Committee of Shandong Jiaotong Hospital and the guardians of all subjects signed informed consent forms. Exclusion criteria included 1) prior history of severe allergic reactions; 2) any severe psychiatric disorder; 3) seizures within the past 6 months; 4) autism caused by active epilepsy, cerebrovascular diseases, or brain trauma; 5) Severity of illness (SI) of Clinical Global Impression (CGI) scale evaluated as “normal” or “borderline mentally ill” or “mildly ill”; 6) moderate or severe extrapyramidal symptoms or tardive dyskinesia; 7) severe self-injury behavior; 8) active systemic or severe focal infections such as human immunodeficiency virus (HIV) and hepatitis; 9) autoimmune diseases; 10) severe pulmonary and hematological diseases, malignancy, or hyp immunity; 11) other treatments that could affect the safety, efficacy, and/or evaluation of stem cell therapy.

### Cell preparation and transplantation

The CBMNC and UCMSC were provided by Shenzhen Beike Biotechnology Co., Ltd. Fresh human cord blood and umbilical cord were obtained from informed healthy donors in accordance with the sterile procurement guidelines established by the hospital in conjunction with the National Ministry of Health. After collection, each sample was tested for communicable diseases, including hepatitis B, hepatitis C, HIV, cytomegalovirus and syphilis, as well as the enzyme alanine aminotransferase, and then transferred for cell preparation in GMP laboratories.

Cord blood was diluted with saline (2:1) and 30 mL diluted blood was then added to 15 mL Ficoll and centrifuged (750 g for 22 min). Mononuclear cells were collected and washed twice in saline. Contaminating erythrocytes were lysed with lysis buffer comprised of injection grade water. Cell density was adjusted to  $2 \times 10^6$ /mL and seeded into Dulbecco's modified Eagle's medium/nutrient mixture F-12 culture medium with bFGF and epidermal growth factor at a concentration of 20 ng/mL. Culture media was mixed with 2% v/v B-27 Stem Cell Culture Supplement. Cells were cultured at 37°C with saturated humidity and 5% CO<sub>2</sub> by volume and harvested for clinical application after 4-7 days of cultivation. The final CBMNC product contained 0.2-1.0% CD34<sup>+</sup> cells as determined by flow cytometry.

The umbilical cord was rinsed twice in normal saline and the cord blood was removed during this process. The washed cord was cut into 2-3 cm pieces and then bluntly dissected to obtain Wharton's jelly. The Wharton's jelly was cut into 1-4 mm<sup>3</sup> pieces, floated in a flask with low glucose Dulbecco's modified Eagle's medium containing serum substitute supplement, and incubated at 37°C in a humidified atmosphere consisting of 5% CO<sub>2</sub>. The medium was replaced every 3-5 days after the initial plating. Well-developed colonies of fibroblast-like cells appeared after 12-14 days, at which time the cells were trypsinized and passaged into a new flask for further expansion. UCMSCs harvested from passage 2 were used in this study and flow cytometry results showed that  $\geq 95\%$  of those cells expressed CD29, CD73, CD90, and CD105, while the expression of CD45, CD34, CD14, CD79, and HLA-DR was 2% or less. The capacity of the UCMSCs to differentiate into adipogenic and osteogenic lineages was identified and a soft agar cloning assay and tumorigenicity experiments with UCMSCs in mice showed no carcinogenicity.

To ensure the quality of the CBMNCs and UCMSCs, cell growth was regularly monitored during the culture period, and all of the inspection information was recorded accordingly, including test results for sterility, mycoplasma and endotoxin ( $\leq 0.5$  EU/mL). Any

contaminated cell suspensions or unhealthy cells were eliminated upon discovery. The finished cell product incorporated a final cell count as requested, cell viability ( $\geq 85\%$ ) determined by trypan blue testing and a sterility test.

After extensive discussion to answer all questions, written informed consents were obtained from each subject's guardian before initiating the scheduled treatments. With support from the accumulated clinical experience of our exploratory stem cell treatments (Sun et al., 2010; Yang, et al., 2010, 2011), the subjects recruited in the CBMNC and Combination groups received four cell transplantations at an interval of 5-7 days. Approximately  $2 \times 10^6$ /kg CBMNCs and  $1 \times 10^6$ /kg UCMSCs were infused with normal saline intravenously (20 mL) and/or intrathecally (2 mL), respectively, per treatment. The patients received two CBMNC intravenous and intrathecal infusions each, followed by two UCMSC intrathecal injections.

### Enzyme-linked immunosorbent assay (ELISA)

A 2 mL CSF sample was collected from each subject before each intrathecal injection. The samples were centrifuged at 3000 rpm for 10 min at 4°C. The supernatant was aliquoted and stored at -70°C. Levels of HGF, BDNF, NGF and bFGF in the supernatant were determined using an ELISA kit (USCN Life Sciences Inc., Wuhan, China), according to the manufacturer instructions.

### Statistical analysis

All data are reported as means  $\pm$  SD and were analyzed using the SPSS 10.0 software. One-way analysis of variance (ANOVA) with post-hoc F- and Q-tests was performed for comparisons. A P value less than 0.05 was considered statistically significant.

### RESULTS

ELISA was used to measure the levels of HGF, BDNF, NGF and bFGF in the CSF of patients after CBMNC or UCMSC transplantation (Table 1). HGF, BDNF and NGF levels in the CSF were significantly increased after transplantation ( $P < 0.05$ ). There were no significant changes in bFGF levels in the CSF after transplantation ( $P > 0.05$ ).

**Table 1.** Levels of HGF, BDNF, NGF and bFGF in the cerebrospinal fluid of subjects (means  $\pm$  SD, N = 20).

Time	HGF (ng/L)	BDNF (ng/L)	NGF (ng/L)	bFGF (ng/L)
1 week after first treatment	22.76 $\pm$ 10.27	16.73 $\pm$ 9.35	19.36 $\pm$ 12.03	21.03 $\pm$ 12.92
1 week after second treatment	136.12 $\pm$ 17.28*	135.46 $\pm$ 15.86*	134.62 $\pm$ 11.07*	24.57 $\pm$ 12.33
1 week after third treatment	203.38 $\pm$ 26.33**	146.03 $\pm$ 22.83**	141.63 $\pm$ 12.38**	34.48 $\pm$ 13.27

\* $P < 0.05$ , vs 1 week after second treatment and 1 week after first treatment. \*\* $P < 0.05$ , vs 1 week after third treatment and 1 week after first treatment.

Treatment safety was evaluated by complete blood count, liver and renal function tests, serum glucose, lipid profile, and immunology tests including immunoglobulin (Ig) A/G/M, complement C3/C4 and T-cell subsets, and tests for HIV, syphilis and HBV at baseline (pre-treatment) at 4, 8, and 24 weeks after the first transplantation. There were no significant changes in these indices for all subjects. There were four cases of low-grade fever, which subsided without medical intervention.

## DISCUSSION

It had long been assumed that neurons have a limited ability to regenerate. However, the discovery of neural stem cells brought with it the promise of new therapeutic approaches for the treatment of diseases of the central nervous system. As one potential source of neural stem cells, mesenchymal stem cells (MSCs) are multi-potent stem cells derived from the mesoderm and have been found in the bone marrow, adipose tissue, umbilical cord tissue, and placenta. MSCs secrete neurotrophic factors and, when transplanted, can promote the secretory activity of host cells. Neurotrophic factors regulate neuronal proliferation and differentiation, and provide support and nutrients to neurons. Transplantation of MSCs promoted the production of brain-derived neurotrophic factor (BDNF) and NGF, both of which are required for neuronal survival, differentiation and myelination (Chang et al., 2011; Lopatina et al., 2011). Several mechanisms have been proposed to explain why transplanted MSCs restore neuronal function. First, MSCs secrete neurotrophic factors. For example, MSCs cultured *in vitro* secrete BDNF and NGF into the culture media. Transplantation of MSCs increased the levels of these factors in injured areas of a middle cerebral artery occlusion (MCAO) mouse model and in the CSF of a traumatic brain injury model (Facchiano et al., 2002; Walker et al., 2009). Second, MSCs promote the self-restoration of host cells. MSCs regulate the cellular microenvironment by secreting growth factors and promoting the production of neurotrophic factors by glial cells, which may promote progenitor/precursor proliferation, migration, and differentiation in the adult brain. For example, injection of bFGF and NGF into the brain promotes the proliferation, migration, and differentiation of progenitor/precursors (Bachstetter et al., 2008). Neurotrophic factors reduce apoptosis in the perinatal zone, stimulate the proliferation of endogenous cells in the subventricular zone, and promote neuronal regeneration, dendrite formation, signal transduction, and the release of neurotransmitters (Lisahlolm, 1997; Li et al., 2002). Third, MSCs promote angiogenesis. It has been shown that after bone marrow stromal cell transplantation, the secretion of VEGF was promoted in the ischemic boundary zone after stroke (Chen et al., 2003).

While the pathophysiology of autism remains poorly defined, accumulating data suggest that one potential etiology may involve immune dysregulation (Onore et al., 2012). Extensive data indicate that an abnormal immune system, including active neuroinflammation in the brain, elevated pro-inflammatory cytokine profiles, dysfunction of immune cells and presence of autoimmunity, are all directly related to increased impairments in behavior. Studies demonstrated an ongoing neuroinflammatory process with marked activation of microglia and astroglia in the cerebral cortex, white matter and cerebellum of individuals with autism (Vargas et al., 2005). A unique proinflammatory cytokine profile in autistic patients has been documented in the cerebrospinal fluid, including a marked increase in macrophage chemoattractant protein-1 (Vargas et al., 2005), and in the peripheral plasma, such as significantly elevated levels of interleukin (IL) -1 $\beta$ , IL-8 and IL-12p40 (Ashwood et al., 2011b). Additional studies found altered function in immune cell subsets (Enstrom et al., 2009; Ashwood et al., 2011a), leading to an inappropriate or ineffective immune response to pathogen challenge in autism. Various autoantibodies responding to proteins in the central nervous system have been detected in the children with autism, which could link autism with an autoimmune process rather than an externally triggered immune reaction (Vojdani et al., 2004; Singer et al., 2006; Ashwood et al., 2006). Collectively, these data suggest that immune dysfunction is not only a symptom/co-morbidity factor but is also indicative of an underlying pathophysiological process, so that targeting this pathology and modifying neuroimmune reactions may be

productive from a therapeutic perspective. However, few clinical trials of anti-inflammatory drugs have aimed to correct the immune dysregulation and ongoing neuroinflammation in autism. Due to their known ability to alter immune responses, MSCs may offer a novel therapeutic solution to ameliorate the immune abnormalities apparent in some children with autism (Shi et al., 2011; De Miguel et al., 2012). MSCs have profound immunoregulatory properties and are currently being investigated as a novel cellular immunomodulatory and anti-inflammatory agent in numerous clinical trials (Kaplan et al., 2011). It has been shown that MSCs can reduce the proliferative capacity of T cells, B cells, NK cells, DC and neutrophils, and modulate a variety of immune cell functions, including cytokine secretion and cytotoxicity of T cells and NK cells, B cell maturation and antibody secretion, DC maturation and activation, as well as antigen presentation (Le Blanc and Ringdén, 2007; De et al., 2012). MSCs can secrete a plethora of growth factors, anti-inflammatory cytokines and immunomodulatory mediators, such as indoleamine 2,3-dioxygenase, prostaglandin E2 (PGE2), nitric oxide, histocompatibility leucocyte antigen-G, transforming growth factor- $\beta$ , interferon- $\gamma$ , hepatocyte growth factor (HGF), IL-6, IL-10 and heme oxygenase-1 (Shi et al., 2011; Soleymaninejadian et al., 2012).

Several studies have shown that cerebral hypoperfusion is associated with many core symptoms of autism (Freeman et al., 2004; Madduri et al., 2009). Generalized brain hypoperfusion, peaking in the frontal and prefrontal regions, was observed in children with autism and is associated with cognitive and neuropsychological deficits (Freeman et al., 2004; Madduri et al., 2009). In addition, decreased cerebral perfusion, especially in the temporoparietal area, has been linked to cognitive impairment, such as language deficits, impairment of cognitive development and object representation and abnormal perception and responses to sensory stimuli (Freeman et al., 2004; Madduri et al., 2009). Inadequate perfusion resulting in brain tissue hypoxia not only causes neuronal apoptosis and necrosis, but also led to abnormal brain tissue metabolism and an accumulation of pathological levels of neurotransmitters (Freeman et al., 2004; Madduri et al., 2009). Therapeutically targeting cerebral ischemia and resulting hypoxia may be an alternative therapeutic approach for autism treatment (Freeman et al., 2004; Madduri et al., 2009). Therapeutic angiogenesis promoted by systemic administration of cord blood CD34<sup>+</sup> stem cells to overcome ischemia has been experimentally demonstrated *in vitro* and in animal models. It has been shown that endothelial progenitor cells contained in the CD34<sup>+</sup> cell population enriched in CBMNCs, have the capacity to trigger angiogenesis in ischemic tissue (Freeman et al., 2004; Madduri et al., 2009). The circulating CD34<sup>+</sup> progenitors in the CBMNC population, having the potential for endothelial development, were recruited to injury sites and developed into new endothelial cells to either repair the injured endothelial wall or sprout new vascular structures (Freeman et al., 2004; Madduri et al., 2009). Moreover, human CD34<sup>+</sup> cells and hematopoietic precursors can secrete numerous angiogenic factors, such as vascular endothelial growth factor (VEGF), HGF, and insulin-like growth factor-1 (IGF-1) (Freeman et al., 2004; Madduri et al., 2009). CBMNC therapies have been successfully translated into preclinical applications for functional recovery in various ischemic animal models through the enhancement of angiogenesis around the site of degeneration (Freeman et al., 2004; Madduri et al., 2009). Given the potency of cord blood CD34<sup>+</sup> cells at promoting angiogenesis in ischemic areas, CBMNCs may be useful for the improvement of the cerebral hypoperfusion and hypoxia that has been suggested to occur in the brains of individuals with autism (Freeman et al., 2004; Madduri et al., 2009).

A variety of growth factors and neurotrophic factors regulate neuronal differentiation,

survival, and regeneration. Transplantation of umbilical cord blood mononuclear cells (CBMNCs) and umbilical cord-derived mesenchymal stem cells (UCMSCs) showed therapeutic efficacy in patients with autism based on the index of Childhood Autism Rating Scale (CARS), Clinical Global Impression (CGI) scale, and Aberrant Behavior Checklist (ABC) (Lv et al., 2013). In this study, we observed significantly increased levels of NGF, BDNF and HGF in the CSF of patients with autism after therapy using transplantation of CBMNCs and UCMSCs. Further studies will be required to identify additional mechanisms by which transplantation of CBMNCs and UCMSCs achieves therapeutic efficacy in autism.

### Conflicts of interest

The authors declare no conflict of interest.

### REFERENCES

- Ashwood P, Wills S and Van de Water J (2006). The immune response in autism: a new frontier for autism research. *J. Leukoc. Biol.* 80: 1-15. <http://dx.doi.org/10.1189/jlb.1205707>
- Ashwood P, Corbett BA, Kantor A, Schulman H, et al. (2011a). In search of cellular immunophenotypes in the blood of children with autism. *PLoS One* 6: e19299. <http://dx.doi.org/10.1371/journal.pone.0019299>
- Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, et al. (2011b). Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. *Brain Behav. Immun.* 25: 40-45. <http://dx.doi.org/10.1016/j.bbi.2010.08.006>
- Bachstetter AD, Pabon MM, Cole MJ, Hudson CE, et al. (2008). Peripheral injection of human umbilical cord blood stimulates neurogenesis in the aged rat brain. *BMJ Neurosci.* 9: 22. <http://dx.doi.org/10.1186/1471-2202-9-22>
- Chang L, Chen Y, Li J, Liu Z, et al. (2011). Cocaine- and amphetamine-rescued transcript modulates peripheral immunity and protects against brain injury in experimental stroke. *Brain Behav. Immun.* 25: 260-269. <http://dx.doi.org/10.1016/j.bbi.2010.09.017>
- Chen J, Zhang ZG, Li Y, Wang J, et al. (2003). Intravenous administration of human bone marrow stromal cells induces angiogenesis in the ischemic boundary zone and stroke in rats. *Circ. Res.* 92: 692-699. <http://dx.doi.org/10.1161/01.RES.0000063403.31163.8D>
- Chen N, Kametani S, Newcomb J, Hudson J, et al. (2007). Trophic factor induction of human umbilical cord blood cells *in vitro* and *in vivo*. *J. Neural Eng.* 4: 129-145. <http://dx.doi.org/10.1088/1741-2560/4/2/013>
- Chen SH, Chang FM, Tsai J, Huang KF, et al. (2005). Resuscitation from experimental heatstroke by transplantation of human umbilical cord blood cells. *Crit. Care Med.* 33: 1377-1383. <http://dx.doi.org/10.1097/01.CCM.0000161266.20056.89>
- De Miguel MP, Ferrás-Julián S, Blázquez-Martínez A, Pascual CY, et al. (2012). Immunosuppressive properties of mesenchymal stem cells: advances and applications. *Curr. Mol. Med.* 12: 574-591. <http://dx.doi.org/10.2174/156652412800619950>
- Enstrom AM, Lit L, Onore CE, Gregg JP, et al. (2009). Altered gene expression and function of peripheral blood natural killer cells in children with autism. *Brain Behav. Immun.* 23: 124-133. <http://dx.doi.org/10.1016/j.bbi.2008.08.001>
- Facchiano F, Fernandez E, Mancarella S, Maira G, et al. (2002). Promotion of regeneration of corticospinal tract axons in rats with recombinant vascular endothelial growth factor alone and combined with adenovirus coding for this factor. *J. Neurosurg.* 97: 161-168. <http://dx.doi.org/10.3171/jns.2002.97.1.0161>
- Fan CG, Zhang QJ, Tang FW, Han ZB, et al. (2005). Human umbilical cord blood cells express neurotrophic factors. *Neurosci. Lett.* 380: 322-325. <http://dx.doi.org/10.1016/j.neulet.2005.01.070>
- Filipek PA, Accardo PJ, Baranek GT, Cook EH, Jr., et al. (1999). The screening and diagnosis of autistic spectrum disorders. *J. Autism Dev. Disord.* 29: 439-484. <http://dx.doi.org/10.1023/A:1021943802493>
- Freeman RS, Burch RL, Crowder RJ, Lomb DJ, et al. (2004). NGF deprivation-induced gene expression: after ten years, where do we stand? *Prog. Brain Res.* 146: 111-126. [http://dx.doi.org/10.1016/S0079-6123\(03\)46008-1](http://dx.doi.org/10.1016/S0079-6123(03)46008-1)
- Habich A, Jurga M, Markiewicz I, Lukomska B, et al. (2006). Early appearance of stem/progenitor cells with neural-like characteristics in human cord blood mononuclear fraction cultured *in vitro*. *Exp. Hematol.* 34: 914-925. <http://dx.doi.org/10.1016/j.exphem.2006.03.010>

- Kaplan JM, Youd ME and Lodie TA (2011). Immunomodulatory activity of mesenchymal stem cells. *Curr. Stem Cell Res. Ther.* 6: 297-316. <http://dx.doi.org/10.2174/157488811797904353>
- Kuan WL and Barker RA (2005). New therapeutic approaches to Parkinson's disease including neural transplants. *Neurorehabil. Neural Repair* 19: 155-181. <http://dx.doi.org/10.1177/1545968305277219>
- Le Blanc K and Ringdén O (2007). Immunomodulation by mesenchymal stem cells and clinical experience. *J. Intern. Med.* 262: 509-525. <http://dx.doi.org/10.1111/j.1365-2796.2007.01844.x>
- Lee MW, Moon YJ, Yang MS, Kim SK, et al. (2007). Neural differentiation of novel multipotent progenitor cells from cryopreserved human umbilical cord blood. *Biochem. Biophys. Res. Commun.* 358: 637-643. <http://dx.doi.org/10.1016/j.bbrc.2007.04.181>
- Li Q, Chen CF, Wang DY, Lü YT, et al. (2015). Transplantation of umbilical cord blood mononuclear cells increases levels of nerve growth factor in the cerebrospinal fluid of patients with autism. *Genet. Mol. Res.* 14: 8725-8732. <http://dx.doi.org/10.4238/2015.July.31.21>
- Li Y, Chen J, Chen XG, Wang L, et al. (2002). Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery. *Neurology* 59: 514-523. <http://dx.doi.org/10.1212/WNL.59.4.514>
- Lindholm D (1997). Neurotrophic factors and neuronal plasticity: is there a link? *Adv. Neurol.* 73: 1-6.
- Lopatina T, Kalinina N, Karagyaur M, Stambolsky D, et al. (2011). Adipose-derived stem cells stimulate regeneration of peripheral nerves: BDNF secreted by these cells promotes nerve healing and axon growth de novo. *PLoS One* 6: e17899. <http://dx.doi.org/10.1371/journal.pone.0017899>
- Lv YT, Zhang Y, Liu M, Qiuwaxi JN, et al. (2013). Transplantation of human cord blood mononuclear cells and umbilical cord-derived mesenchymal stem cells in autism. *J. Transl. Med.* 11: 196-205. <http://dx.doi.org/10.1186/1479-5876-11-196>
- Madduri S, Papaloizos M and Gander B (2009). Synergistic effect of GDNF and NGF on neuronal survival and elongation *in vitro*. *Neurosci. Res.* 65: 88-97. <http://dx.doi.org/10.1016/j.neures.2009.06.001>
- Mitchell S, Brian J, Zwaigenbaum L, Roberts W, et al. (2006). Early language and communication development of infants later diagnosed with autism spectrum disorder. *J. Dev. Behav. Pediatr.* 27(10): S79-S78. <http://dx.doi.org/10.1097/00004703-200604002-00004>
- Neuss S, Becher E, Wöltje M, Tietze L, et al. (2004). Functional expression of HGF and HGF receptor/c-met in adult human mesenchymal stem cells suggests a role in cell mobilization, tissue repair, and wound healing. *Stem Cells* 22: 405-414. <http://dx.doi.org/10.1634/stemcells.22-3-405>
- Onore C, Careaga M and Ashwood E (2012). The role of immune dysfunction in the pathophysiology of autism. *Brain Behav. Immun.* 26: 383-392. <http://dx.doi.org/10.1016/j.bbi.2011.08.007>
- Park M and Lee YH (2013). Cord blood transplantation for the treatment of acute leukemia. *Chin. Med. J. (Engl)* 126: 761-767.
- Shi M, Liu Z and Wang FS (2011). Immunomodulatory properties and therapeutic application of mesenchymal stem cells. *Clin. Exp. Immunol.* 164: 1-8. <http://dx.doi.org/10.1111/j.1365-2249.2011.04327.x>
- Singer HS, Morris CA, Williams B, Yoon DY, et al. (2006). Antibrain antibodies in children with autism and their unaffected siblings. *N. Engl. J. Med.* 354: 150-158. <http://dx.doi.org/10.1016/j.jneuroim.2006.05.025>
- Soleymanejadian E, Pournazeri K and Samadian E (2012). Immunomodulatory properties of mesenchymal stem cells: cytokines and receptors. *Am. J. Reprod. Immunol.* 67: 1-8. <http://dx.doi.org/10.1111/j.1600-0897.2011.01069.x>
- Sun L, Wang D, Liang J, Zhang H, et al. (2010). Umbilical cord mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus. *Arthritis Rheum.* 62: 2467-2475.
- Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, et al. (2005). Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann. Neurol.* 57: 67-81. <http://dx.doi.org/10.1002/ana.20315>
- Vojdani A, O'Bryan T, Green JA, McCandless J, et al. (2004). Immune response to dietary proteins, gliadin and cerebellar peptides in children with autism. *Nutr. Neurosci.* 7: 151-161. <http://dx.doi.org/10.1080/10284150400004155>
- Walker PA, Shah SK, Harting MT and Cox CS, Jr. (2009). Progenitor cell therapies for traumatic brain injury: barriers and opportunities in translation. *Dis. Model. Mech.* 2: 23-38. <http://dx.doi.org/10.1242/dmm.001198>
- Yang WZ, Zhang Y, Wu F, Min WP, et al. (2010). Safety evaluation of allogeneic umbilical cord blood mononuclear cell therapy for degenerative conditions. *J. Transl. Med.* 8: 75-80.
- Yang WZ, Zhang Y, Wu F, Zhang M, et al. (2011). Human umbilical cord blood-derived mononuclear cell transplantation: case series of 30 subjects with hereditary ataxia. *J. Transl. Med.* 9: 65.