

# Neural stem cell secretome and its role in the treatment of neurodegenerative disorders

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Neurological diseases in the central nervous system are mostly characterized by the failure of endogenous repair to restore tissue damage and salvage lost function. Currently, studies have shown that neural stem cell transplantation provides a good therapeutic effect on neurological diseases. For this reason, neural stem cell transplantation has been explored as a cell replacement therapy. Although transplanted cells can replace cells lost during or post central nervous system injury, many studies have shown that this mechanism is insufficient as most of these newly formed cells fail to integrate and eventually die. Although it was initially thought that neural stem cell could only replace lost cells, recent experiments have shown that transplanted neural stem cell can also play bystander roles such as neuroprotection and immune regulation, promote tissue repair by preventing tissue damage, interfere with pathogenic processes, or by rescuing endogenous nerve cells. However, compelling evidence has raised concerns about this bystander effect, which can be caused by several biologically active molecules (collectively known as the secretome) produced by neural stem cells. These results also raise the possibility of the neural stem cell secretome as a potential candidate for neural stem cell transplantation therapies based on the bystander effect. A better understanding of the molecules and mechanisms of this effect is of critical importance for neural stem cell-based therapies. This review aims to discuss the function and application of neural stem cell secretome in the treatment of neurodegenerative disorders.

## Keywords

Neuroplasticity; bystander effect; secretome; neurodegenerative disorders; neural stem cell

## 1. Introduction

Neural stem cells (NSC) are heterogeneous cells with mitotic activity, self-renewal, and multipotency, which drive neurogenesis and gliogenesis throughout the life of most mammals, including humans. When a neural injury occurs, NSCs have been shown to retain the capacity to migrate to damaged areas, promote functional and structural tissue repair and generate both neurons and glia with complex gene expression patterns and differ-

ences in space and time (Adams and Morshead, 2018; Göritz and Frisén, 2012). Recent technical advances allow NSCs to be obtained from three sources (Tang et al., 2017), including direct extraction from primary tissues (Belenguer et al., 2016), differentiation from pluripotent stem cells (Banda and Grabel, 2016) and transdifferentiation from somatic cells (Shahbazi et al., 2017).

The various characteristics of NSC transplantation offers great promise for the treatment of a wide variety of diseases, such as neurological disease (including spinal cord injury (Suzuki et al., 2017; Zhu et al., 2018), traumatic brain injury (Duan et al., 2016; Koutsoudaki et al., 2016), epilepsy (Lippert et al., 2017; Shetty, 2014), cerebral palsy (Tan et al., 2014; Zheng et al., 2012) and cerebrovascular diseases, including stroke (Lee et al., 2010). Ischemic and hemorrhagic stroke (Mozaffarian et al., 2016), is a leading worldwide cause of disability and death (Yu et al., 2018). A recent study has shown that transplanted NSCs could improve neurological deficits and reduced the severity of ischemic stroke by restoring endogenous striated spinous neurons, as well as by inhibiting inflammation and glial scar formation (Bacigaluppi et al., 2009). The results of other experiments have shown that rats show good functional performance in neurological tests 2-8 weeks after NSCs transplantation, which further demonstrates that exogenous NSCs could be used for functional recovery after hemorrhagic stroke (Lee et al., 2010). On this evidence, NSC transplantation is undoubtedly a promising therapy for the treatment of cardiovascular and neurological diseases.

## 2. The therapeutic mechanism of neural stem cell transplantation

Current methods for NSC transplantation in animal experiments and clinical applications include intravenous or intrathecal injections (Wang et al., 2017), intracerebral injection (Sullivan and Armstrong, 2017), and stereotactic (Li et al., 2013) and intraventricular injection (Griffin et al., 2015). As Chu et al. (2004) have shown, intravenously transplanted human NSCs can migrate to and differentiate within lesion sites to improve neural function, NSC transplantation has also been shown by the rotary table, limb position and turning ability tests to improve sensorimotor performance. Another study has shown that stereotactic transplanted NSCs can differentiate into neuronal (50%) and astroglial (15%) cells, which may then provide the replacement of cells where they are lost following injury (Kelly et al., 2004).

Although the therapeutic mechanisms of cell replacement for transplanted NSCs have led to a large number of studies, until now, they have increasingly suggested that direct replacement of missing cells may not be the main mechanism of NSC transplantation (Bernstock et al., 2017; Marsh and Blurton-Jones, 2017). Several studies have shown this mechanism to be largely inadequate because most of the newly formed cells fail to integrate and eventually die (Arvidsson et al., 2002; Thored et al., 2010). Much research has shown that the beneficial role of NSCs may be attributed to other biological characteristics. For example, transplanted neural progenitor cells (NPCs) can play a bystander effect of neural protection and immune regulation in addition to the role of cell replacement, thus promoting tissue repair by prevention of tissue damage, interfering with pathogenic processes, or saving endogenous nerve cells (De Feo et al., 2012). Additionally, different studies have confirmed other bystander effects induced by NSCs, such as ameliorating damage to neurophysical structures, immunoregulation (Kim et al., 2018), neuroprotection (Cui et al., 2015; Pang et al., 2017), neurotrophication (Bernstock et al., 2017; Marsh and Blurton-Jones, 2017) and angiogenesis (Boese et al., 2018), as well as by having an endogenous impact in disease models (Jablonska et al., 2016).

Although NSC transplantation is undoubtedly a promising therapy for neurological disease, their limited persistence/survival in the harsh microenvironment of ischemic regions rich in reactive oxygen species means that the clinical transformation of this method remains elusive (Bernstock et al., 2017). However, the NSC secretome may not only help circumvent this difficult problem but also provide a consistent "on-demand" solution. There is, therefore, a promising therapeutic prospect held out by the NSC secretome in both replacement and auxiliary cell transplantation.

### 3. NSC secretomes

In addition to the abilities of self-renewal and differentiation into all neural cell lineages, NSCs secrete through the autocrine/paracrine pathway, a large number of bioactive compounds that play important therapeutic roles. Studies have shown that in pathological conditions, NSCs can regulate the microenvironment through a paracrine mechanism. This plays an important role in maintaining the characteristics of stem cells and directional differentiation. NSCs can also perform intercellular responses through paracrine signaling (Bollini et al., 2013; Drago et al., 2013). It is bioactive compounds, such as chemokines, cytokines, early inflammatory cytokines, growth factors, nutritional factors, stem cell regulatory factors, extracellular vesicle and other similar molecules that are generally referred as the NSC secretome (Fig. 1), which is considered important in regulating several critical biological processes, such as cell survival, proliferation and differentiation, immune regulation, anti-apoptosis and stimulation of adjacent cells in tissues (Cossetti et al., 2012; Skalnikova et al., 2011). Up until the present, studies have shown that neurotrophic factors derived from NSCs play important roles in cell cycle regulation (Einstein and Ben-Hur, 2008), cell survival (Marsh and Blurton-Jones, 2017), development and adult differentiation (Hyman et al., 1991; Numakawa et al., 2017).

Drago et al. (2013) have shown that significant repair of damaged brains can be achieved by injecting biological agents secreted

by stem cells, rather than by directly inserting stem cells for cell replacement.

Further, brain-derived neurotrophic factor (BDNF) has been shown to have some success in ameliorating symptoms in several studies of preclinical models of Huntington's disease (Gharani et al., 2007; Giral et al., 2009). Trials are underway to treat Alzheimer's disease with nerve growth factor (NGF) (Allen et al., 2013) which is administered either as injected proteins (Tian et al., 2012), released locally by implanted NGF-secreting cells (Tuszynski et al., 2005) or by introducing an adeno-associated virus-based gene delivery vector that encodes for human NGF (Bishop et al., 2008). Additionally, stem cell-secreted factors have been used in several clinical studies (Drago et al., 2013), and the clinical trial of autologous mesenchymal stem cell secreting factor for the treatment of amyotrophic lateral sclerosis has been completed (ClinicalTrials.gov Identifier: NCT01051882), as has intraventricular infusion (Nutt et al., 2003) or putaminal infusion (Lang et al., 2006) of glial cell-derived nutrient factor (GDNF) which has recently been shown to be an effective treatment for Parkinson's disease and is currently undergoing clinical trials (Allen et al., 2013).

In addition to endogenous NSCs, transplanted NSCs can also secrete a series of factors such as chemokines, cytokines, growth factors, and stem cell regulators, which exert immune regulation and nutritional support after reaching a lesion site (Kokaia et al., 2012). Following a study of an amyotrophic lateral sclerosis disease model which found that transplanted NSCs secrete neurotrophic factors that promote the survival and regeneration of motor neurons, Kim et al. (2018) found that the secretome of transplanted NSCs also plays a role in immunoregulation and neuroprotection (Kim et al., 2018; Xu et al., 2006).

## 4. The function of NSC secretome neuroprotection

### 4.1 Protect neurons

Despite the initial perception that NSC transplantation only served to replace lost cells, recent experimental studies have shown that transplanted NSCs also exert additional bystander effects such as the neuroprotection that promote tissue repair (Aharonowicz et al., 2008). To verify this characteristic of NSCs, Liang et al. (2014) removed the neural stem cell from a normal culture medium to acquire neural stem cell-conditioned medium to exclude the effect of cell substitution. Results showed that continuous administration of conditioned medium in rats with spinal cord injury increases bridging between the corticospinal tract and intermediate neurons reduces neuron apoptosis, and promotes the recovery of motor function. Other findings show that the conditioned medium of NSCs may provide a better neuroprotective effect than the normal medium. This was attributed to neurotrophic factors such as NGF, GDNF, and a vascular endothelial growth factor (VEGF) secreted by NSCs (Lee et al., 2017). Additionally, another studies have found that in primary and secondary neurodegenerative disorders such as Huntington's disease (Ryu et al., 2004), spinal cord injury (Ziv et al., 2006) and stroke (Chu et al., 2004), the main mechanism of NSCs in prevention of neuronal programmed cell death is also mediated by NGF and BDNF, ciliary neurotrophic factor (CNTF) and GDNF, which are secreted by transplanted NSCs/NPCs.

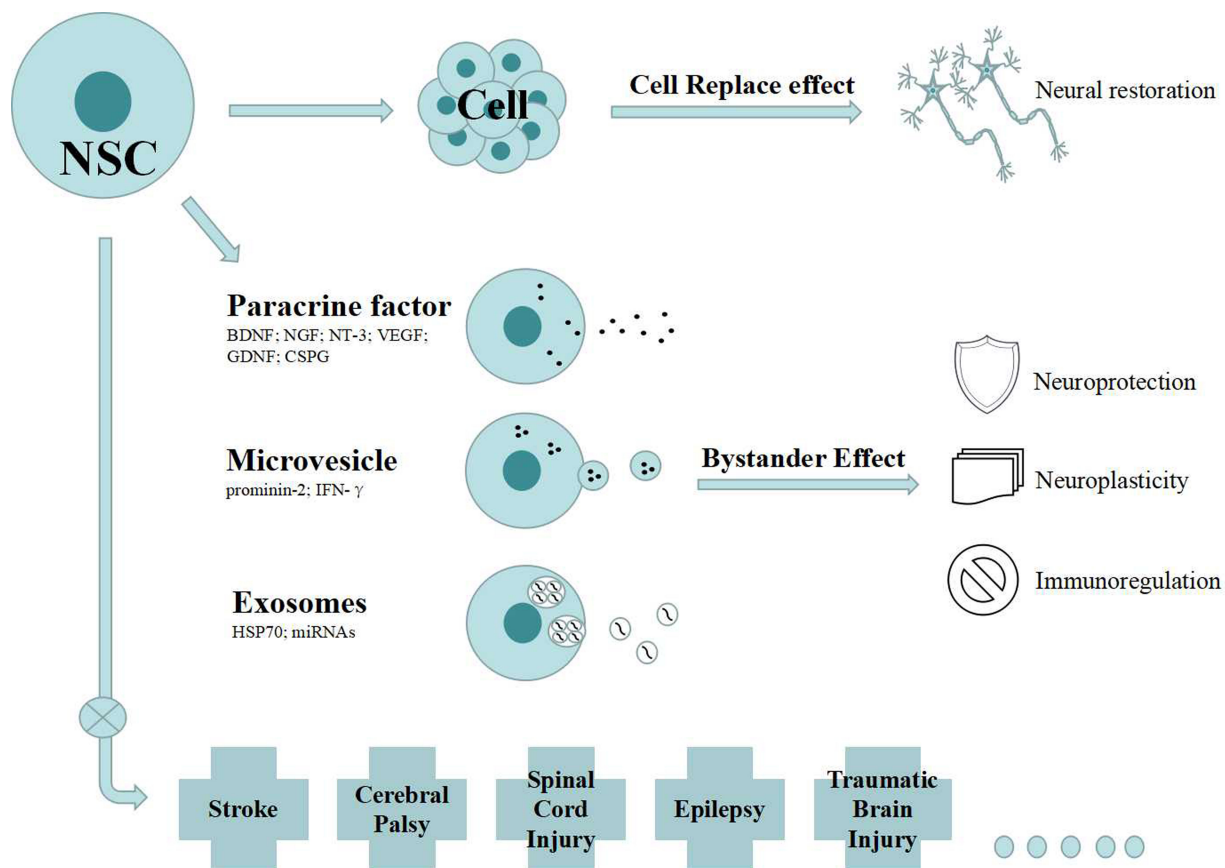


Figure 1. Bystander effect generated by NSC secretome. NSC transplantation for the treatment of neurodegenerative disorders, including spinal cord injury, traumatic brain injury, epilepsy, cerebral palsy, and stroke, among others. The main mechanism for NSC transplantation may not only be by direct replacement of missing cells but also through the bystander effect, which includes neuroprotection, neuroplasticity, immunoregulation by NSC secretomes (paracrine factor, microvesicles, and exosomes).

#### 4.2 Protect oligodendrocytes

It has been shown that CNTF secreted by NPCs can participate in the survival and differentiation of oligodendrocyte progenitor cells (OPCs) (Marriott et al., 2010). Further, in a demyelinating disease model, transplanted NSCs induce the proliferation of OPCs and promotes myelin regeneration by secreting platelet-derived growth factor (PDGF)-AA and basic fibroblast growth factor (FGF-2) (Einstein et al., 2009). The protective effects of these two factors on OPCs have been widely demonstrated: a growing number of research groups have demonstrated that FGF-2 (Chen et al., 2015; Raff et al., 1983) and PDGF (Bertold et al., 1999; Watzlavik et al., 2013) promote the survival and proliferation of OPCs *in vitro* and (Barres et al., 1992; Bertold et al., 1999).

#### 4.3 Other effects

The neuroprotective effects of transplanted undifferentiated human fetal NSCs in ischemic stroke are attributed not only to the secretion of nutrient factors (i.e., VEGF) but also to the re-expression of developmental molecules, i.e., Slit-a family of secreted extracellular matrix proteins (Andres et al., 2011).

In addition to factors, the secretome also contains many extracellular vesicles, including exosomes and microvesicles. It is well known that exosomes are carriers of intercellular communication, transporting and transferring microRNA between cells (Camussi et al., 2010; Lotvall and Valadi, 2007). Currently, re-

searchers have identified miRNA in exosomes of NSCs using second-generation sequencing technology and found 113 miRNA types, many of which are relevant for neural regeneration, neuroprotection and neural plasticity (Stevanato et al., 2016). Until now, various miRNA has been found to have repair and treatment effects on central nervous system (CNS) injuries, such as stroke, traumatic brain injury, and spinal cord injury (Faravelli and Corti, 2017; Sun et al., 2018). Some studies have shown that cortical neurogenesis is increased by targeting exosomal miR-124 after stroke (Yang et al., 2017).

Further, another study has shown downregulated miR-134 by electroacupuncture enhances expression of LIM domain kinase and increases synaptic-dendritic plasticity following ischemic stroke in a rat model (Schratt et al., 2006). Since their discovery and development, new therapeutic approaches to stroke are being based on NSC-derived exosomal miRNAs (Zhang et al., 2018). Furthermore, other researchers have determined proteins of the human NSCs exosome by tandem mass spectrometry and identified 103 proteins, including HSP70 and the transferrin receptor (Kang et al., 2008). Among them, HSP70 is considered a protective agent by its inhibition of oxidative stress that affects a variety of neurological diseases, including craniocerebral injury, chemical-toxic nerve injury, and Alzheimer's disease (Cossetti et al., 2014).

## 5. The function of NSC secretome: Neuroplasticity

### 5.1 Nerve regeneration

NSCs transplantation has been shown to increase the expression of VEGF, platelet reactive protein 1/2, and cysteine-rich acidic proteins in the brain of stroke patients. These are key factors in promoting dendritic growth, axonal plasticity, and axonal transport (Andres et al., 2011). In an experimental model of spinocerebellar ataxia type 1, transplanted NSCs have been shown to induce electrochemical coupling with damaged neurons through the formation of gap junctions, thereby preventing local cell damage (Jäderstad et al., 2010).

### 5.2 Angiogenesis

Since stroke may damage the brain vascular system, angiogenesis may help restore neural function. Relevant studies have shown that CTX0E03 cells, a human NSC line, express angiogenic factors *in vitro*, and may promote angiogenesis through paracrine factors and direct physical interactions. Additionally, *in vivo* results show a significant increase in microvessels in the rodent brain where cells were implanted. Accordingly, NSC-based therapy may provide significant benefits to stroke patients by upregulation of angiogenesis in ischemic lesions (Caroline et al., 2013; Stroemer et al., 2009). Moreover, a further study demonstrated for the first time that NSC-secreted VEGF is required to enhance the angiogenesis signaling pathway *in vivo* (Horie et al., 2015). The authors suggest that NSCs affect not only endothelial cells but also other key components of the vascular system in a VEGF-dependent manner, such as by enhancing the expression of  $\beta$ -dystroglycan, an extracellular matrix adhesion protein rich in perivascular astrocyte endings and expressed in peripheral and endothelial cells (Milner et al., 2008; Zaccaria et al., 2001).

## 6. The function of NSC secretome: Immunoregulation

One study has confirmed that NSCs transplantation exhibits an immunoregulatory role in spinal cord injury. This is mediated by decreased production of M1-type macrophages after injury and increased production of M2-type macrophages, which phagocytize disintegration products and secrete anti-inflammatory factors, thus inhibit the inflammatory response (Cusimano et al., 2012).

Additionally, another study shows that NPC transplantation not only inhibits T-cell proliferation (Einstein et al., 2003) but also promotes apoptosis of infiltrating T cells (Pluchino et al., 2005). This may be achieved by two mechanisms: one is the induction of expression of death receptor ligands, such as FasL, Trail, and Apo3L; the other is by promoting the production of soluble molecules associated with mitochondrial-mediated apoptosis, such as GDNF, interferon- $\gamma$ , and leukemia inhibitory factor (De Feo et al., 2012).

Interestingly, although VEGF is generally thought to be pro-inflammatory, increasing literature has confirmed the anti-inflammatory properties of VEGF secreted by NSCs. Some studies have found that VEGF not only inhibits the development of antigen-presenting dendritic cells and T cells but also suppresses their activation (Gabrilovich et al., 1996; Ohm et al., 2003). VEGF may also influence macrophage proliferation in a dose-dependent manner since Manoonkitiwongsa et al. (2006) found that low-dose

VEGF treatment of ischemic brain injury reduces macrophage number, while high-dose VEGF increases macrophage density. Of note, other experiments confirmed that VEGF is key for the immunosuppressive effects of NSCs and may be involved in altering the integrity of the blood-brain barrier (Horie et al., 2015).

## 7. Conclusion

In conclusion, NSC secretomes contain various factors and extracellular vesicles that play a role in neuroprotection, neuroplasticity, and immunoregulation of the nervous system. Although the paracrine mechanism of NSCs is presently not well understood, current research shows that the NSC secretome has considerable therapeutic significance and prospects. Compared with the direct use of NSCs, the secretome may have many unique advantages, such as easy access by ultrafiltration centrifugation or other similar methods, longer storage time, simpler transportation mode, and body lesions can be simulated to stimulate the production of more specific paracrine products. Overall, more understanding about the role of NSC secretomes and their clinical benefits in neurological disease could contribute to the discovery and development of new therapeutic approaches.

## Abbreviations

ALS: amyotrophic lateral sclerosis; BDNF: brain-derived neurotrophic factor; CNS: central nervous system; CNTF: ciliary neurotrophic factor; CSPG: chondroitin sulfate proteoglycan; GDNF: glial cell-derived nutrient factor; HSP70: heat shock protein 70; NGF: nerve growth factor; NPCs: neural progenitor cells; NSCs: neural stem cell; NT-3: neurotrophin 3; VEGF: vascular endothelial growth factor.

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## Conflict of Interest

The authors declare no conflicts of interest.

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