The potential of neural transplantation for brain repair and regeneration following traumatic brain injury

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Abstract

Traumatic brain injury is a major health problem worldwide. Currently, there is no effective treatment to improve neural structural repair and functional recovery of patients in the clinic. Cell transplantation is a prospective therapy for TBI as transplanted cells may differentiate into region-specific cells and integrate into the host tissue to replace the lost cells in the injured brain. This review article summarized recent development in cell transplantation studies for post-traumatic brain injury brain repair with varying types of cell sources. It also discussed the potential of neural transplantation to repair/promote recovery of the injured brain following traumatic brain injury.

Key Words: traumatic brain injury; stem cells; neural transplantation; regeneration; functional recovery

Introduction

Following traumatic brain injury (TBI), the primary and secondary injury-induced neural tissue loss is permanent. As the mature mammalian brain has limited capacity to repair and replace the damaged neurons, neural transplantation is a prospective therapy for TBI as transplanted cells may differentiate into region-specific cells and integrate into the host tissue to replace the lost cells in the injured brain. Additionally, transplanted cells could provide trophic support to the host tissue to facilitate regeneration. Over the past decades, researchers have explored a wide array of cell sources for neural transplantation. These cells include embryonic stem cells isolated from fetal or embryonic tissue, mesenchymal stromal cells such as bone marrow stromal cells and umbilical cord cells, adult neural stem cells (NSCs) and, more recently, induced pluripotent stem cells (iPSCs) (Figure 1). The following sections will discuss the application of these cell types in the setting of TBI.

Embryonic Stem Cells

Embryonic stem (ES) cells are pluripotent stem cells that have unlimited capacity of self-renewal and can give rise to cells of all three primary germ layers. Due to their high plasticity, ES cells are the idea cell source for neural transplantation. When transplanted into normal or damaged CNS, human ES cells can differentiate, migrate and are capable of making innervations (Hentze et al., 2007). Thus far, ES cells derived from human or mouse fetal brains have been tested as transplantation cell source for TBI treatment in animal studies in different TBI models with varying results reported (Riess et al., 2002; Shear et al., 2004; Wennersen et al., 2004; Boockvar et al., 2005).

An earlier study showed that NSCs from human ES cells isolated from fetal brain were capable of survival for an extended period of time up to 6 weeks, migrating to the contralateral cortex and differentiating into the injured brain following a cortical contusion injury (Wennersen et al., 2004). Gao et al. (2006) have reported that NSCs from human ES cells survive and differentiate to neurons after transplantation into the injured brain when examined at 2 weeks after cell injection, and the injured animals with cell transplantation had improved cognitive functional recovery. Shear et al. (2004) assessed the long-term survival, migration, differentiation and functional significance of NSCs derived from mouse fetal brain after transplantation into the injured brain up to 1 year post-transplantation. They found that the injured animals receiving transplants showed significant improvement in motor and spatial learning functions, and the transplanted cells migrated widely in the injured brain, with the majority of transplanted cells expressing NG2, an oligodendrocyte progenitor cell marker but not neuronal markers (Shear et al., 2004). Post-TBI neural transplantation of immortalized fetal ES-derived NSCs (C17.2 cells) has also shown improved motor function with the transplanted cells surviving for up to 13 weeks and differentiation into mature neurons and glial cells (Riess et al., 2002; Boockvar et al., 2005). In vitro modified ES cells either pre-differentiated into mature
neurons expressing neurotransmitters or over-expressing growth factors such as brain cell line-derived growth factor (GDNF), brain derived neurotrophic factor (BDNF), and human multi-neurotrophins showed beneficial effects when transplanted into the injured animals by promoting motor, and cognitive improvements concomitant with better graft survival and neuronal differentiation (Bakshi et al., 2006; Becerra et al., 2007; Ma et al., 2012; Blaya et al., 2015).

Taken together, these data suggest that post-TBI transplantation using ES-derived cells can restore motor and cognitive functions of the injured animals. However, the beneficial effect of the transplanted cells may be associated with neural trophic effect of the transplanted cells rather than directly neural replacement as long term survival and neuronal differentiation is rather limited. Skardelly et al. (2011, 2014) found that following transplantation of pre-differentiated human fetal ES cells, either focally into the injured rat brain or systemically following a controlled cortical injury, a transient increase of angiogenesis and reduced astrogliosis were observed together with improved long-term motor functional improvement, reduced brain injury lesion volume and increased neuronal survival in the border zone of the lesion; however, graft differentiation was rare, and some of the beneficial effects of cell transplantation were diminished at 12 weeks after transplantation. Further studies are needed to investigate how to prolong the survival of transplanted cells and improve their integration into functional neural circuitry by modulating the injured host environment. Caution must be taken when working with multipotent ES cells as undifferentiated ES cells have the risk of potential tumor formation (Riess et al., 2007).

**Adult NSCs**

Recent findings show that the mature mammalian CNS harbors multipotent stem cells capable of differentiation into a variety of specialized cells throughout life (Lois and Alvarez-Buylla, 1993; Gage et al., 1998). In the adult mammalian CNS, the NSCs/neural progenitor cells (NPCs) are primarily confined to the subventricular zone (SVZ) surrounding the lateral ventricle and the dentate gyrus (DG) of the hippocampus (Altman and Das, 1965; Lois and Alvarez-Buylla, 1993). Aside from these major neurogenic regions, adult neurogenesis in rodents has also been reported in other regions in the CNS including the striatum, substantia nigra, cortex and spinal cord (Weiss et al., 1996; Palmer et al., 1999; Lie et al., 2002). These adult derived NSCs express low levels of major histocompatibility complex antigens (Klassen et al., 2003) and due to their low expression of the major histocompatibility complex antigens (MHC Class II) (Le and Ringden, 2005). In addition, these cells produce high level of growth factors, cytokines and extracellular matrix molecules that could have potential neurotrophic or neuroprotective effects in the injured brain. As a matter of fact, all studies using BMSCs for neural transplantation have demonstrated that the beneficial effects of BMSCs are attributed to their neurotrophic, neuroprotective and anti-inflammatory effects rather than direct cell replacement (Li and Chopp, 2009; Zhang et al., 2013).

In humans, multipotent stem/progenitors cells have been identified and successfully isolated from various regions of adult human brain including the hippocampus, SVZ, neocortex, and subcortical white matters from neurosurgical resection tissues (Kukekov et al., 1999; Roy et al., 2000; Arsenijevic et al., 2001; Brunet et al., 2002, 2003; Windrem et al., 2002; Nunes et al., 2003; Richardson et al., 2006). This has raised the possibility of using these cells as autologous cell sources for transplantation therapies. Indeed, Brunet et al. (2005) demonstrated that adult monkey NSCs/NPCs, derived from cortical biopsies, survived for at least 3 months and displayed a neuronal phenotype after re-implantation into the normal or ibotenic acid excitotoxic lesioned motor cortex of the donor brains (Brunet et al., 2005). These cells may also be possible to restore the anatomy and function of the injured CNS as shown in a study after grafting adult human NSCs/NPCs into the demyelinated rat spinal cord (Akiyama et al., 2001).

To date, very few studies have attempted to examine the behavior of adult derived human NSCs/NPCs in the injured mature CNS. Ostorn et al. (2007) recently reported that a small portion (4 ± 1%) of adult human NSCs/NPCs can survive for 16 weeks after transplantation into the posterior periventricular region in normal adult rats or rats with hippocampal CA1 ischemic injury. Although the results of this study are promising, questions remain whether these cells become anatomically and functionally integrated into the injured brain and whether the proportions of surviving cells can be increased by transplanting NSCs/NPCs at a different developmental stage.

**Bone Marrow Stromal Cells (BMSCs)**

Due to ethical and immunological concerns as well as the risk of tumorigenesis, the translational value of using ES cells for clinic application is limited. Autologous transplantation of NSCs isolated from neurosurgical removed brain tissue from TBI patients is an attractive strategy; however, thus far the success of long term cell survival and functional outcomes of these cells in the treatment of experimental TBI is rather limited. Due to these limitations, adult derived mesenchymal cells, particularly BMSCs, have received much attention.

BMSCs are undifferentiated cells with mixed cell population including stem and progenitor cells. These cells can be easily isolated from the mononuclear fraction of bone marrow from patients and be expanded in culture without ethical and technique concerns. Another advantage of considering BMSCs for cell transplantation is the low antigenicity due to their low expression of the major histocompatibility complex antigens (MHC Class II) (Le and Ringden, 2005). In addition, these cells produce high level of growth factors, cytokines and extracellular matrix molecules that could have potential neurotrophic or neuroprotective effects in the injured brain. As a matter of fact, all studies using BMSCs for neural transplantation have demonstrated that the beneficial effects of BMSCs are attributed to their neurotrophic, neuroprotective and anti-inflammatory effects rather than direct cell replacement (Li and Chopp, 2009; Zhang et al., 2013).
The potential of BMSCs for treating TBI has been extensively assessed in experimental TBI models. Cells were delivered either focally to the injured brain, or systemically through intravenous or intra-arterial injections at the acute or sub-acute phase after TBI and significant reduction of neurological deficits including motor and cognitive deficits was reported. For example, intracranial injection of rat BMSCs into the brain region adjacent to the brain lesion site or intravenous injection of cells at 24 hours after a controlled cortical contusion injury in rats were reported and they found that the injured animals had improved sensory motor functional improvement (Lu et al., 2001; Mahmood et al., 2001, 2003). When human BMSCs were combined with collagen scaffolds and transplanted into the injury cavity at 4 or 7 days following TBI, animals had significantly improved sensorimotor and spatial learning functions, together with reduced brain lesion volume and enhanced focal brain angiogenesis (Lu et al., 2007; Xiong et al., 2009). Co-transplantation of BMSCs with collagen scaffolds has also shown improved cell survival and neurite outgrowth and better functional recovery (Guan et al., 2013). The effect of BMSCs in improving sensorimotor function of injured animals was reported even when delivered at 2 months following TBI (Bonilla et al., 2009). Further studies have demonstrated that the beneficial effort of BMSCs in the injured brain is due largely to their production of bioactive factors which facilitates the endogenous plasticity and remodeling of the host brain (Li and Chopp, 2009). Although low number of BMSCs was found in the injured brain expressing neuronal or glial markers (Mahmood et al., 2001, 2003, 2006), no study has sufficiently demonstrated that MSCs can be fully differentiated into functional neurons in vivo. Taken together, extensively experimental studies have demonstrated the beneficial effects of BMSCs in the injured brain and highlight the potential of using BMSCs for TBI treatment in clinic.

Other Potential Types of Cells and Strategies for Cell Replacement Therapy
Apart from the aforementioned stem cells, in recent years researchers have explored several other type of stem or stem like cells for TBI application. Thus far, published data have reported that human amnion-derived multipotent progenitor cells significantly attenuated axonal degeneration, improved neurological function, and protected brain tissue morphology of the injured rats (Chen et al., 2009; Yan et al., 2013). Intravenous administration of human adipose-derived stem cells or culture medium into a controlled cortical impact rat model significantly improved motor and cognitive functions and reduced focal tissue damage and hippocampal cell loss (Tajiri et al., 2014).

The human umbilical cord blood is an abundant source of multiple stem cells, including hematopoietic stem cells, mesenchymal stem cells, unrestricted somatic stem cells, and embryonic-like stem cells. These cells can be easily harvested without ethical controversy and can be an attractive source of stem cells for brain repair. Thus far, studies have shown that these cells can survive at the injury sites and promote survival of local host neurons in ischemic and spinal cord injury animal models (Sun and Ma, 2013). In a small scale of clinic trial of using these cells for treating TBI patients, it was reported that patients treated with umbilical cord stem cells had improved neurological function and self-care compared to the control group with no cell transplantation (Wang et al., 2013). Similar to BMSCs, the reported beneficial effect of post-TBI transplantation with these cells is likely due to the neurotrophic effect of the transplanted cells, as direct neuronal differentiation and long term survival were rarely observed.

Compared to the aforementioned MSCs, peripheral blood derived MSCs may be a more approachable cell source. In a recent study, post-TBI transplantation of a subpopulation of human peripheral blood derived MSCs following in vitro priming resulted in improved cognitive function, decreased apoptosis of injured cortical brain tissue, and increased production of neurotrophic factors while some transplanted cells migrated to the site of injury with extended survival and neuronal differentiation at 3 months after injection into...
the lateral ventricle (Nichols et al., 2013).

Recent development of somatic cell reprogramming which generates induced pluripotent stem cells (iPSCs) provides prospects for novel neural replacement strategies. Human iPSCs possess dual properties of unlimited self-renewal and the pluripotent potential to differentiate into multi-lineage cells without ethical concerns. More importantly, patient-specific iPSCs can serve as autologous cell source for transplantation without encountering graft rejection. These unique properties of iPSCs have raised the widespread hope that many neurological diseases including TBI might be cured or treated. Thus far rapid progress has been made in the field of reprogramming, however, the optimal source of somatic cells used for applications in neurological disorders has not yet been identified.

Compared to direct cell transplantation using exogenous cell source, in situ neuronal generation/cell replacement at the site of injury could be of greater potential for brain repair. Recent studies have reported in vivo reprogramming of astrocytes into neuroblasts by SOX2 overexpression (Niu et al., 2013), or by inhibiting Notch1 signaling in astrocytes (Magnusson et al., 2014). Moreover, reactive astrocytes in the cortex of injured or diseased mouse brain can be converted into functional neurons by overexpression of transcription factor NeuroD1 (Guo et al., 2014). These studies suggest that direct reprogramming of reactive glial cells to functional neurons at the site of brain injury could be a more attractive strategy for post-TBI brain repair.

Conclusion and Perspectives

Extensive studies have shown the prospective of brain repair through cell replacement strategy using varying types of stem cells. However, to successfully repair and regenerate the injured brain with stem cells, many challenges must be overcome. One major challenge is the focal microenvironment of the site of injury. Following TBI, primary brain damage together with secondary tissue loss induced by ischemia, excitotoxicity, oxidative stress and inflammation creates a hostile environment preventing the survival and integration of the transplanted cells. Thus far, ample studies have supported the notion that the in vivo fate of transplanted cells is regulated by the intrinsic properties of grafted cells and the local environmental cues in the host. These challenges must be overcome in experimental TBI studies before moving forward stem cell therapies for treating the injured brain clinically.

References


