NEURAL REGENERATION RESEARCH
May 2014, Volume 9, Issue 9
www.nrronline.org

INVITED REVIEW

Stem cells in neuroinjury and neurodegenerative disorders: challenges and future neurotherapeutic prospects

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doi:*10.4103/1673-5374.133129*
http:*//www.nrronline.org/*

Accepted: *2014-04-15*

Abstract

The prevalence of neurodegenerative diseases and neural injury disorders is increasing worldwide. Research is now focusing on improving current neurogenesis techniques including neural stem cell therapy and other biochemical drug-based approaches to ameliorate these disorders. Unfortunately, we are still facing many obstacles that are rendering current neurotherapies ineffective in clinical trials for reasons that are yet to be discovered. That is why we should start by fully understanding the complex mechanisms of neurogenesis and the factors that affect it, or else, all our suggested therapies would fail since they would not be targeting the essence of the neurological disorder but rather the symptoms. One possible paradigm shift is to switch from neuroprotectant therapies towards neurodegeneration/neurorestorative approaches. In addition, other and our laboratories are increasingly focusing on combining the use of pharmacological agents (such as Rho-associated kinase (ROCK) inhibitors or other growth factors (such as brain-derived neurotrophic factor (BDNF)) and stem cell treatment to enhance the survivability and/or differentiation capacity of transplanted stem cells in neurotrauma or other neurodegeneration animal models. Ongoing stem cell research is surely on the verge of a breakthrough of multiple effective therapeutic options for neurodegenerative disorders. Once, we fully comprehend the process of neurogenesis and its components, we will fully be capable of manipulating and utilizing it. In this work, we discuss the current knowledge of neuroregenerative therapies and their associated challenges.

Key Words: *neural stem cells; neurodegeneration; neuroinjury; TBI; rho-associated kinase (ROCK) inhibitor; BDNF; growth factors; stem cell therapy*

Mouhieddine TH, Kobeissy FH, Itani M, Nokkari A, Wang KKW. Stem cells in neuroinjury and neurodegenerative disorders: challenges and future neurotherapeutic prospects. Neural Regen Res. 2014;9(9):901-906.

Introduction

The prevalence of neurodegenerative diseases is increasing worldwide. That is not only due to increased incidences of direct or indirect injury to the central nervous system (CNS) but also due to the increase in the percentage of the aging population. Incurable neurodegenerative disorders such as Alzheimer's disease (AD), traumatic brain injury (TBI), Parkinson's disease (PD) and multiple sclerosis (MS) have reached a staggering prevalence of around 5.2 million (Hebert et al., 2013), 1.7 million (Faul et al., 2010), 500 thousand (National Institute of Neurological Disorders and Stroke, 2014a) and 250–350 thousand (National Institute of Neurological Disorders and Stroke, 2014b) cases, respectively, in the USA alone. With these relatively high numbers of cases and our current deficit in effective therapeutic modalities that cure those disorders, it is imperative that we find new neurotherapeutic targets to prevent the increasing morbidities or at least ameliorate them. For the past few decades, researchers have been interested in the realm of stem cells and the prospect of using them in an attempt of curing diseases that entail organ, tissue and cellular degeneration. Even with the exponential increase in our knowledge of stem cell formation and manipulation, we are still far from fully understanding the intricate mechanisms in which these intriguing cells function. That is apparent in studies that do not get a 100% remission rate, or encounter relapses or side effects in animal models or human subjects in clinical trials following a failure of stem cells transplant. Yet, this field remains to be promising, and with the progress we are at, we are not far from a major breakthrough in the field of stem cells in general and neuronal stem cells in particular.

The endogenous pool of neural stem cells

The greatest challenge of stem cell research is to be able to apply stem cell therapy in neurodegenerative diseases, due to several reasons. For one, the nervous system remains till

today the least understood system of all, even with most research concentrating on it for the past few decades. Another factor is that neuronal cells are one of the very few body cells that remain almost post-mitotic. A third reason is the scarcity and restriction of the endogenous pool of neuronal stem cells (Maldonado-Soto et al., 2014). Until recently, it was believed that neurogenesis is a distinct characteristic of embryogenesis and very early life and that neurons of adulthood are post-mitotic cells with no endogenous pool of neuronal and glial stem cells. Earlier studies have shown that through human surgical samples, *in vitro* neurons could be generated (Kirschenbaum et al., 1994; Pincus et al., 1998). These were later followed by post-mortem human studies that detected the presence of neuroblast markers and migration indices (Bedard and Parent, 2004; Curtis et al., 2007). Now, we know that adult neurogenesis is possible *via* a pool of progenitor stem cells. There are stem cells in the subventricular zone (SVZ) of the lateral ventricles, which propagate to the olfactory bulb, and in the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG), which aid in the maintenance of spatial memory formation and cognition (Kempermann, 2012; Kohman and Rhodes, 2013; Sawada and Sawamoto, 2013).

Through the incorporation of reagents such as doublecortin and bromo-deoxy-brimidine, it was found that during adult neurogenesis, the stem cell pool undergoes extensive proliferation before transforming into glial and neural progenitor cells, which mature within 3–4 weeks (van Praag et al., 2002). It has been determined that during neurogenesis, quiescent progenitor cells are activated and asymmetrically divide into amplifying neural progenitor cells, which would in turn transform into post-mitotic, migratory neuroblasts or glioblasts (Encinas et al., 2011). It is the alterations in this delicate process that underlie or augment the pathogenesis of many of the neurodegenerative diseases where replacement of diseased or injured neurons is reduced or even totally blocked. Furthermore, it has been found that cognitive decline may start during the second decade of human life (Salthouse, 2009) and, with aging, the proliferation rate of the endogenous neural stem cell population of rodents decreases by 50–80% (Ahlenius et al., 2009) and they may even reach a terminal astrocytic differentiation of their neural progenitors (Encinas et al., 2011), indicating that there is some kind of biological clock controlling neurogenesis.

Aging, environmental factors and neurogenesis

What scientists are trying to do now is to find an effective neuronal replacement therapy, but what we really need to do is to better understand the mechanism of aging and disease progression. Cell replacement therapy is not working since the replenished stem cells are being destroyed *via* unknown disease mechanisms, and thus the only way to prevent this is by understanding these mechanisms in order to know how to protect our endogenous pool and the administered cells. It has been discovered that, with aging, the number of migrating neuroblasts decreases in the SVZ and periventricular white matter of humans (Taylor et al., 2013). Moreover, the culprits behind the aging process of neuronal stem cells include cyclin-dependent kinase (CDK) inhibitors and telomere shortening (Mandal et al., 2011), and a dysregulation of certain factors, or their receptors, such as tumor necrosis factor-α (TNF-α) (Tropepe et al., 1997), epidermal growth factor (EGF) (Pastrana et al., 2009), fibroblast growth factor (FGF) (Frinchi et al., 2008) and Notch delta (Imayoshi et al., 2010). Thus, one interesting way of preserving the potential of our endogenous pool of neural stem cells is by inhibiting CDK inhibitors and telomere shortening (by enhancing telomerase activity) and maybe even finding a way to prevent the dysregulation of the different factors that are negatively affecting neurogenesis. Yet, the downside of inhibiting CDK inhibitors and enhancing telomerase activity is the increased possibility of developing an oncogenic phenotype within the stem cell population (Mandal et al., 2011). Nevertheless, the process of aging remains to be the highlight of continuous investigation in the hopes that, one day, we could reach a breakthrough in reversing, inhibiting or at least diminishing this process.

When studying stem cells, there are many factors to consider, and undoubtedly, more factors will emerge as we delve deeper into this newly explored realm. One of the most important factors affecting neurogenesis are environmental factors, and that could be translated into daily life style activities (smoking, diabetes, stress and exercise) in which many studies have established their pivotal role in controlling neurogenesis (Bruijnzeel et al., 2011; Farioli-Vecchioli et al., 2014; Loi et al., 2014; Ramos-Rodriguez et al., 2014). These factors could contribute to epigenetic alterations that would translate into differential signaling pathways (such as activated NFkB), which may in turn recede the process of neural regeneration (Koo et al., 2010).

Microglia and neurogenesis

We are still unable to fully conceive the mechanisms employed by microglial cells; different studies have shown that microglia may have trophic, toxic, phagocytic and developmental functions within the brain, as they alternate between an activated and deactivated state (Marin-Teva et al., 2004; Nakajima and Kohsaka, 2004; Monier et al., 2006; Sierra et al., 2010; Verney et al., 2010; Antony et al., 2011; Cunningham et al., 2013; Ueno et al., 2013). To better understand the way microglia function, recent studies have ablated certain genes, such as *Cx3cr1-cre* and *Cx3cr1-creER*, in order to inhibit individual functions and investigate their potential role (Kim et al., 2010; Goldmann et al., 2013; Wolf et al., 2013). Such methods of ablating genes, *via* small interfering RNA (siRNA) for example, may present promising techniques for better understanding the interrelation of microglia and neurogenesis.

RNA and neurogenesis

Another domain to consider for improving our neural stem cell therapeutic modalities is the field of microRNAs (miRNA). Studies have shown that these few-base pair molecules may have a huge impact on neuronal development, such as the miR-29b that represses several pro-apoptotic genes during early neural development and differentiation (Kole et al.,

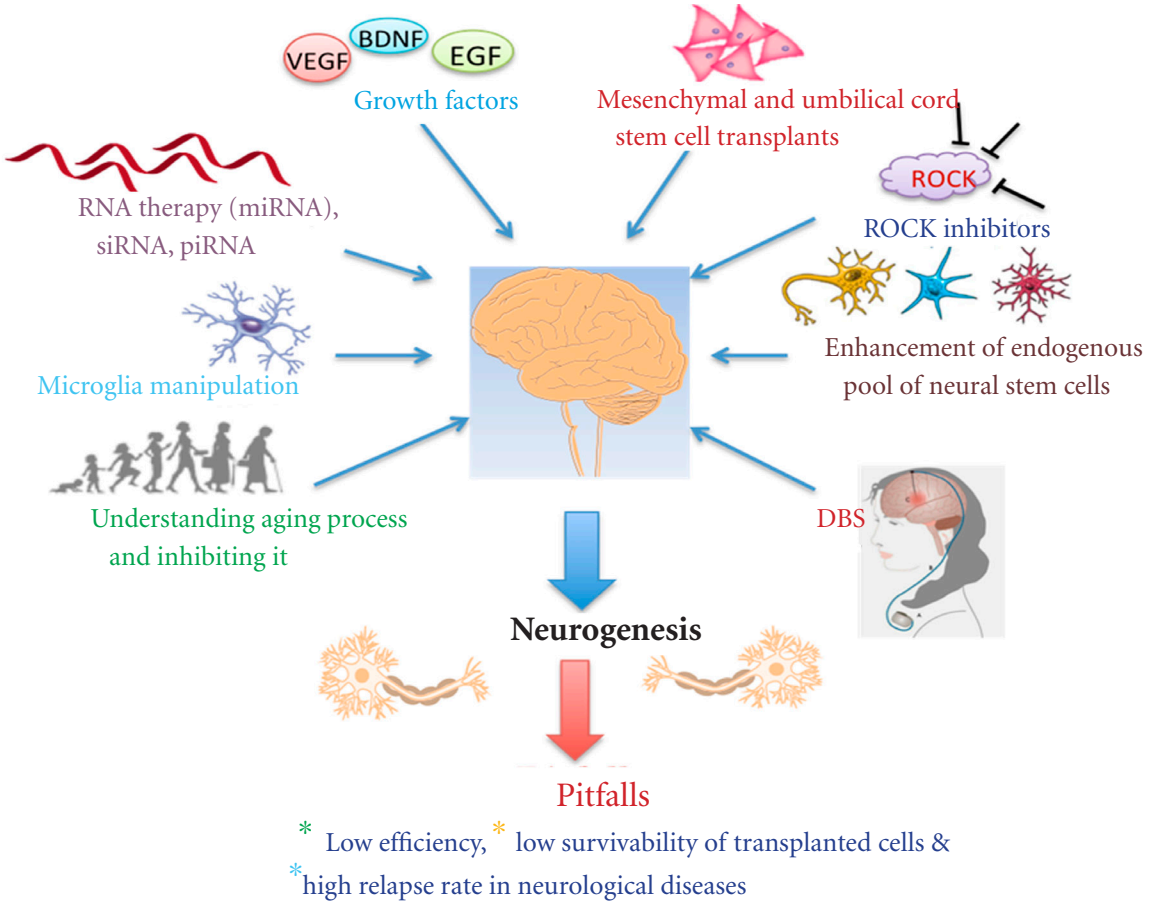

Figure 1 A schematic diagram showing the major methods currently employed for applying and understanding neurogenesis, and the major pitfalls.
VEGF: vascular endothelial growth factor; BDNF: brain-derived neurotrophic factor; EGF: epidermal growth factor; ROCKs: Rho-associated kinases; DBS: deep brain stimulation.

2011) and miR-124 that enhances neuronal differentiation during embryonic development (Makeyev et al., 2007; Visvanathan et al., 2007). Furthermore, a new set of non-encoding RNA, called piRNA, was recently discovered to be responsible for suppressing transposable elements and it has been recently shown to be expressed in the CNS (Lee et al., 2011). In addition to that, it has been shown that piRNAs are up-regulated by serotonin, and in turn, lead to the down-regulation of the CREB2 gene, thereby enhancing memory formation (Rajasethupathy et al., 2012). Another class of RNAs is the long non-coding RNA (lncRNA), which was also recently discovered to be present in the brain and responsible for maintaining neural self-renewal by binding to sites correlated with transcription factors of pluripotency (Ng et al., 2012).

Umbilical cord blood transplants and neurogenesis

Whether it may be a neurodegenerative disease or a TBI, it is important for us to devise an effective way to replace the damaged neurons which are way too much for the relatively small endogenous stem cell population to replenish. In one study, it was found that it is possible to generate *in vitro* phenotypically layer-specific neurons through passaged neurospheres, which are designed to heal damaged areas with functionally compatible neuron subtypes (Cramer et al., 2014). Another method of healing is using umbilical cord blood (UCB), which contains a mixture of stem and progenitor cells. UCB transplants were able to restore sensorimotor abilities of hypoxic rat brains (Geissler et al., 2011) and enhance tissue repair, cognition (de Paula et al., 2012) and neural stem cell proliferation (Wang et al., 2013). Furthermore, it has been shown that autologous intravenous UCB transplantation is a safe technique to use in children with acquired neurological disorders (Buzanska et al., 2006). That is why there are several ongoing clinical trials to test for the safety and efficacy of UCB transplants in curing various neurological disorders. What remains is to devise a successful way to incorporate the neural grafts into the damaged CNS, restore the neural circuitry and ensure the graft's survival. Furthermore, it is important that we become better capable of expanding fetal derived tissue, or UCB, *in vitro* and the transplanted tissue itself, along with promoting the differentiation of the transplanted stem cells. We could even employ deep brain stimulation (DBS), where Encinas et al. (2011) have shown that it increases the number of cells by

69% in the DG of mice by inducing the proliferation of the endogenous pool of neural stem cells. It could be possible that DBS induces the same effect on the endogenous human neural stem cells and even better activate and maintain the transplanted neural tissue.

Topical administration of mesenchymal stem cells for treating TBI

In an experiment in our lab, we devised a new method to utilize mesenchymal stem cells to treat TBI in rats (Lam et al., 2013). Previous methods to administer mesenchymal stem cells led to a limited number of stem cells reaching the site of brain injury (Harting et al., 2009; Walker et al., 2009). These methods included intravenous infusions that scatter cells in various organs, intra-arterial administration that causes microemboli and cerebral ischemia, and direct intracerebral implantation that is invasive and may be fatal. After inducing TBI on the right parietal cortex of rats, we topically administered green fluorescent protein (GFP)-expressing mesenchymal cells, which were isolated from the adipose tissue of rats, along with fibrin for fixation to the left parietal cortex (Lam et al., 2013). Five days later, GFP cells were detected proliferating at the site of application and the site of injury, and in rats without administered fibrin, very few cells were detected at the application site. Furthermore, no GFP-expressing mesenchymal cells were detected in other somatic organs in both control rats and rats with TBI. Moreover, transplanting cells into an uninjured site prevents the death of the transplanted cells due to inflammation following TBI, and provides the cells with the appropriate environment or settling in and proliferating before migrating to the site of injury. Thus, our neurotherapeutic method provides a new and effective way for inducing neurogenesis following TBI, but which needs further research and experimentation for optimization.

ROCK inhibitors and possible combination of stem cell therapy

In our lab, we investigated the possibility of inducing neuronal regeneration through the use of Rho-associated kinases (ROCKs), a class of serine/threonine kinases, which are important mediators of axonal repair in injuries of the CNS (Zhang et al., 2006). We found that ROCK inhibitors promoted neurite outgrowth *via* dramatic cytoskeletal reorganization in PC-12 cell lines, in a time- and dose-dependent manner. Furthermore, we discovered that the effect of ROCK inhibitors was mediated *via* dephosphorylation of cofilin, which when dephosphorylated, binds to actin and enhances depolymerization of actin-associated filaments. Thus, we have shown that ROCK inhibitors could be utilized as a therapeutic technique for treating damaged neurons by promoting their regeneration through axonal repair. However, this technique still needs further investigations to fully understand the mechanisms at hand and to make it as effective as possible in the clinical setting.

ROCK inhibitors have been recently implicated in improving the *in vitro* and *in vivo* survivability and neuronal differentiation capacity of transplanted stem cells. Human embryonic stem cells have been hard to culture, propagate and differentiate since they undergo massive apoptosis following dissociation, but this problem was diminished and cloning efficiency increased from 1 to 27% when using the ROCK inhibitor, Y-27632 (Watanabe et al., 2007). Furthermore, Y-27632 allowed stem cells to differentiate into cortical and basal telencephalic progenitors. Moreover, several studies established novel protocols and found that utilizing Y-27632 increased the survivability and enhanced the recovery of cryopreserved human embryonic stem cells (Martin-Ibanez et al., 2008; Li et al., 2009; Martin-Ibanez et al., 2009; Xu et al., 2010). In another study, it was shown that Y-27632 could inhibit the anti-neurogenesis/neurodifferentiation effect of chondroitin sulfate proteoglycan, which is found in glial scar tissue, on mesenchymal stem cells (Lim and Joe, 2013). Thus, this particular study shows that ROCK inhibitors could be employed as an adjuvant to stem cell transplant to counteract the anti-regenerative effects of factors produced by damaged brain tissue. Further research should be done to discover more pharmacological agents such as Y-27632 that are even more efficient in enhancing the survival and neuronal differentiation of transplanted stem cells.

Growth factors used to enhance stem cell therapy

Growth factors have also been extensively studied and have been found to aid the *in vitro* stem cell culturing and the *in vivo* transplantation process. In one study, it was found that transplanting human umbilical stem cells, transfected with vascular endothelial growth factor (VEGF) and human fibroblast growth factor 2 (FGF2), into an amyotrophic lateral sclerosis (ALS) rodent model led to the transformation of stem cells into astrocytes (Rizvanov et al., 2011). Without VEGF and FGF2, transplanted stem cells differentiated into endothelial cells and microglia. However, when they received signals from dying motor neurons and autocrine signals from these growth factors, they differentiated into astrocytes and contributed to the survival of damaged neurons. Another study also found a way to enhance the transplantation process, where it was also shown that genetically modifying neural stem cells into expressing brain-derived neurotrophic factor (BDNF) allows a more efficient neuronal differentiation and higher survivability with significantly improved motor functions when transplanted into rats with TBI (Ma et al., 2012). Interestingly, it has also been shown that the proliferation rate of *in vitro* SVZ-derived cells is increased *via* transferrin receptor 1 when apotransferrin is added to the culture media (Silvestroff et al., 2013). Such a finding could abolish the fact that we have such a negligible pool of endogenous stem cells, since we can extract some of those cells, proliferate them in culture, and transplant them at a high number to give rapid and effective results. These are just a few of many studies on biological substances and growth factors that may enhance stem cell therapy; it seems only logical that we continue the quest of searching for factors within our bodies that may hold the cure. The cure for neurodegenerative diseases may be inside our bodies after all, but just needs a bit of enhancement.

A nice illustration of all of the above neurotherapeutic interventions is depicted in **Figure 1**.

Conclusion

The field of neural stem cell research in the area of neurodegenerative disorders is highly promising and still in its infancy. However, we have started to pave our way through and are on our way of uncovering many of the secrets of neurogenesis that are still eluding us. As we have discussed, we currently have many options in which to target neurogenesis as a therapeutic technique for neurodegenerative diseases and injuries, but none of which have actually given a long-term effect. The reason for that lies in the fact that we still have not fully grasped the concept of neurogenesis and how it really works. That is why it is imperative that we further investigate and fully understand this fascinating and complicated mechanism before devising new therapeutic modalities or else we might be wasting a lot of time and money on therapies that may never work.

Acknowledgments: *We acknowledge Prof. Poon WS and Dr. Lam PK of the Chinese University of Hong Kong for their scientific input.*

Author contributions: *Mouhieddine TH was the primary writer of the manuscript. Kobeissy FH contributed to generation of the schematics and revising the manuscript. Itani M and Nokkari A contributed to review content compilation and revising the manuscript. Wang KKW contributed to overall conceptualization of the review and revising and improvement of the manuscript.*

Conflicts of interest: *None declared.*

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