Cell Therapy and Structural Plasticity Following Cerebral Ischemia

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1.1 Introduction

Stroke is one of the leading causes of adult disability in the world. For ischemic stroke, the main therapy is the clot lytic agent tissue plasminogen activator, which must be administered within the first 4.5 h (Del Zoppo et al. 2009). As this time frame is limited and depends on the acute detection of clinical symptoms, much research has focused on preventing secondary injury after an initial ischemic or thrombotic event, although clinical translation has been limited (Ginsberg 2008). A new paradigm shift in therapeutic targets for stroke focuses on brain repair, particularly the brain's plasticity—the ability to regenerate synaptic structures and reorganize its functional architecture after injury. This phenomenon is observed in many stroke patients who often initially present with acute loss of motor function but then regain a remarkable degree of functional independence in the weeks or months following the insult. Experimental studies have shown that the surrounding uninjured parenchyma generates new dendrites and axons that can, to some extent, compensate for the functional loss of injured tissue.

Since brain plasticity occurs days to months after stroke, therapies that target such brain repair would significantly open the therapeutic time window of intervention, thus benefiting a significantly larger patient population than current strategies. Growing evidence points to the potential of cell transplantation therapy to promote

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brain plasticity after stroke as will be discussed in this chapter. An assortment of cell types, including brain-, bone marrow-, blood-, and dental pulp-derived progenitors, enhance recovery in experimental models of stroke (Chen et al. 2001a; Darsalia et al. 2011; Guzman et al. 2008a; Hicks et al. 2009; Kelly et al. 2004), reviewed in Bliss et al. (2007), and hemorrhagic stroke (reviewed in Andres et al. 2008b). Due to these early auspicious results, phase I and II clinical trials are now in progress (Andres et al. 2008a; Locatelli et al. 2009; Onteniente and Polentes 2011; Wechsler 2009) where a positive outcome will bring us closer to a much needed new therapeutic strategy for stroke. Understanding the mechanism of action of the transplanted cells will facilitate the successful translation of cell transplantation strategies to the clinic. Here we discuss the effects of transplanted cells on brain plasticity after stroke.

1.2 Anatomical Reorganization as the Basis of Functional Recovery After Stroke

Following an ischemic or hemorrhagic stroke, the immediate devastating neurological deficits on motor and cognitive abilities usually improve within the first few weeks and gradually into the first year (Benowitz and Carmichael 2010). The initial recovery is attributed to the reduction in edema and the inflammatory response. Long-term recovery, however, is most likely due to plastic mechanisms at the synaptic levels that lead to the reorganization of the ischemic penumbra (Benowitz and Carmichael 2010; Dancause 2006; Murphy and Corbett 2009). In humans, functional imaging has revealed compensatory recruitment of areas ipsilateral and contralateral to the stroke site during cognitive tasks (Cramer 2008). This activation pattern becomes more refined to ipsilateral cortical areas with time and correlates with good recovery. Similar results are seen with animal studies (Dijkhuizen et al. 2003; Takatsuru et al. 2009), which suggest this gross remapping is caused by local and long distant changes in axonal sprouting and dendritic arborization (Gonzalez and Kolb 2003; Jones and Schallert 1992).

1.3 Dendritic and Axonal Reorganization After Stroke

Many studies provide evidence for remodeling of dendrites in the ischemic penumbra surrounding the cortical infarct. Chronic changes in dendritic structural plasticity after stroke have been reported with increased contralesional layer V dendritic branching peaking at 18 days post stroke (Jones and Schallert 1992), while ipsilesional layer III branching was decreased (compared with uninjured animals) at 9 weeks post stroke (Gonzalez and Kolb 2003). Exposing animals to an enriched environment after ischemia increased dendritic complexity in the contralateral cortex and enhanced functional recovery (Biernaskie and Corbett 2001), although a causal link between the two was not proven. Brown et al. showed an increase in dendritic spine density and turnover rates in the penumbra area close to the stroke region in the first 2 weeks after stroke (Brown et al. 2009). Interestingly, the increase

in spine density was greatly affected by activity frequency, where restriction of limb use leads to less dendritic sprouting and performance of complex tasks results in significant complex sprouting (Jones et al. 1999).

Axonal regeneration has likewise been demonstrated in different models of ischemic stroke with new projections thought to target areas denervated by the stroke injury (Benowitz and Carmichael 2010). In rodent and primate models of ischemic cortical injury, such sprouting has been observed locally around the infarct area (Carmichael et al. 2001; Conner et al. 2005; Dancause et al. 2005; Li et al. 2010). For example, the rat barrel cortex following an experimental stroke has shown a robust axonal sprouting response that remaps the body representation of the somatosensory cortex (Carmichael et al. 2001). For this experiment, the axonal tracer biotinylated dextran amine (BDA) was administered into the rat whisker barrel (somatosensory) cortex bordering the infarct region 3 weeks after focal strokes revealing new intracortical projections that arose from the ischemic penumbra. In primates after brain injury, the primary motor area also undergoes significant axonal reorganization with circuits of the premotor and somatosensory areas, thus demonstrating that stroke induces axonal sprouting near the injury site and also promotes novel connections with areas distant from the injury (Dancause et al. 2005). In addition to axonal sprouting in the injured cortex, interhemispheric axonal outgrowth from the intact cortex to the injured hemisphere has also been observed after stroke (Carmichael 2008). Moreover, long descending pathways from the cortex to the spinal cord, such as the corticospinal tract that mediates voluntary movements, also reorganize in association with the recovery of limb function in rodents (Liu et al. 2008; Weidner et al. 2001), often with cross midline sprouting from the intact spinal cord to the denervated areas of the injured spinal cord (Chen et al. 2002). Despite measurable brain plasticity after stroke, axonal and dendritic reorganization is limited to a great extent by molecular factors that either inhibit or stimulate growth, and as such, thorough characterization of these molecules will facilitate therapeutic interventions to promote brain rewiring.

1.4 Cell Transplantation Enhances Brain Plasticity After Stroke

Cell transplantation has shown much promise in experimental models of stroke with a diverse array of cell types including brain-, bone marrow-, blood-, and dental pulp-derived progenitors reported to enhance functional recovery after ischemic stroke (Chen et al. 2001a; Darsalia et al. 2011; Guzman et al. 2008a; Hicks et al. 2009; Kelly et al. 2004), reviewed in Bliss et al. (2007), and hemorrhagic stroke, reviewed in Andres et al. (2008a). Despite many preclinical studies showing that cell transplantation can improve recovery from stroke, the mechanisms mediating recovery are less understood. However, emerging evidence suggests that transplanted cells act to enhance endogenous repair mechanisms normally activated in the brain after stroke including brain plasticity (Andres et al. 2011a; Arvidsson et al. 2002; Bliss et al. 2010; Horie et al. 2011; Li et al. 2010; Liu et al. 2008; Ohtaki et al. 2008).

1.4.1 Transplanted Cells Promote Dendritic Plasticity

Fetal-derived neural progenitor cells can enhance dendritic branching in both the ischemic and contralateral hemispheres (Andres et al. 2011a). Compared with vehicle controls, human neural progenitor cell (hNPC)-transplanted rats showed significant enhancement of dendritic branching of Golgi-stained dendrites from layer V cortical pyramidal neurons at 2 weeks post transplantation in both hemispheres (Andres et al. 2011a). Dendritic changes in the contralesional hemisphere had abated at 4 weeks after transplantation; the effects in the ipsilesional hemisphere were sustained. This evidence suggests that dendritic proximity to hNPCs helps sustain branching. Increases in dendritic branching were most significant in basilar dendrites and middle-order branches; however, the significance of this for neuron function remains to be elucidated. The pattern of early dendritic changes in the contralesional cortex followed by a switch to more dominant changes in the ipsilesional cortex at later times is reminiscent of brain remapping results in patients and animals. These remapping studies show that stimulation of the injured limb early after stroke recruits the contralesional cortex and this switches back to the ipsilesional cortex at later time points (Benowitz and Carmichael 2010; Dancause 2006). It would thus be of interest to determine the significance of these dendritic changes to such remapping data.

1.4.2 Transplanted Cells Promote Axonal Rewiring After Stroke

Axonal sprouting occurs after stroke with new projections thought to target areas denervated by the stroke injury (Benowitz and Carmichael 2010). In rodent and primate models of ischemic cortical injury, such sprouting has been observed locally around the infarct area (Carmichael et al. 2001; Conner et al. 2005; Dancause 2006; Li et al. 2010), and interhemispheric axonal outgrowth from the intact cortex to the injured hemisphere has also been observed (Carmichael 2008). BDA axonal tracer studies from our lab and others showed that hNPCs (Andres et al. 2011a; Daadi et al. 2010) and human umbilical cord blood cells (Xiao et al. 2005) enhance interhemispheric cortical sprouting in corticocortical, corticostriatal, and corticothalamic pathways. In addition, hNPCs increased expression of the axonal growth cone protein GAP-43 in the corpus callosum and bilateral cortical hemispheres, with the largest change seen in the peri-infarct cortex (Andres et al. 2011a). However, GAP-43 is not purely a marker of regenerating axons as it is also expressed on nonneuronal cells such as astrocytes and oligodendrocytes (Carmichael 2008); the importance of this for regeneration is not understood. hNPC and mesenchymal stem cell (MSC) transplantation also enhanced stroke-induced remodeling of cortical spinal tract axons originating from the contralesional cortex (i.e., intact corticospinal tract) (Andres et al. 2011; Chen et al. 2002; Liu et al. 2008); such remodeling also included sprouting of the intact corticospinal tract into denervated regions of the spinal cord. Cell-induced changes in both corticospinal tract and transcallosal axonal sprouting statistically correlated with cell-enhanced functional recovery implying that cell-induced axonal plasticity is an important mechanism for stem- cell-induced recovery, although a direct causal link remains to be demonstrated.

Previous reports have demonstrated impairment of axonal transport following stroke (Wakita et al. 2002). Since axonal transport is fundamental to neuronal function and survival, we subsequently investigated the effect of transplanted hNPCs on anterograde axonal transport by measuring amyloid precursor protein (APP) accumulation in axons. We found that hNPCs significantly enhanced recovery of axonal transport following ischemia (Andres et al. 2011). Compared with vehicle controls, hNPC-transplanted rats had significantly fewer APP-positive axons at 3 weeks with further enhancement at 5 weeks. Using SMI312 immunostaining for axons, we also demonstrated more axons in the corpus callosum at 5 weeks in hNPC-treated animals. Moreover, APP accumulation in the corpus callosum negatively correlated with functional recovery in the whisker-paw and cylinder behavioral tests. These data suggest that transplanted hNPCs help improve axonal transport and enhance functional recovery.

This is a significant finding as axonal transport is fundamental to neuron function, not only for proper functioning and survival of existing axons but also for plasticity changes such as axonal sprouting and synaptogenesis. Therefore, hNPC-induced restoration of impaired axonal transport after stroke may not only enhance the function of existing fiber tracts but may also be a key upstream event of hNPC-induced structural plasticity.

1.5 How Do Stem Cells Modulate Brain Plasticity?

There are two mechanisms through which stem cells could modulate plasticity: (1) through secretion of factors that can modulate plasticity events, directly or indirectly, and (2) by integration into host circuits.

1.5.1 Transplanted Cell-Secreted Factors Modulate Dendritic and Axonal Plasticity

Neural progenitor cells and MSCs express many factors known to influence neurite plasticity (Kurozumi et al. 2005; Llado et al. 2004; Wright et al. 2003) including molecules such as neurotrophic factors like vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (Himmelseher et al. 1997; Rosenstein et al. 2003), extracellular matrix molecules like the thrombospondins and secreted protein acidic and rich in cysteine (SPARC) (Au et al. 2007; Liauw et al. 2008; Osterhout et al. 1992), and factors important for neurite growth and guidance during development like Slit (Dancause 2006; Lin and Isacson 2006). Using immunodepletion experiments in a noncontact coculture assay of hNPCs with primary cortical neurons, we found that depletion of thrombospondins 1 and 2 or human VEGF significantly reduced hNPC-induced dendritic branching and length, while neutralization of Slit only affected total dendritic length (Andres et al. 2011). However, depletion of any of the above factors significantly reduced hNPC-mediated axonal outgrowth. In addition, using a microfluidic platform, we were able to show in vitro that neutralization of VEGF—not thrombospondins 1 and 2, SPARC, or Slit—inhibited hNPC-mediated effects on axonal transport. These data demonstrate that hNPC-secreted factors can modulate

neuronal plasticity at least in vitro. Whether these factors are also important for the in vivo effects of hNPCs and MSCs remains to be determined. However, using quantitative polymerase chain reaction analysis, we were able to identify the expression of VEGF and thrombospondins 1 and 2 in transplanted hNPCs in stroke brains 1 week post transplantation (Andres et al. 2011), and we also showed that hNPC-secreted VEGF is necessary for hNPC-induced recovery (Horie et al. 2011), thus suggesting a potential role for these molecules in vivo.

The exact effects of stem-cell-secreted factors on brain plasticity have yet to be elucidated. It is possible that they act through common pathways that affect axonal plasticity, possibly through downregulation of plasticity inhibitors or upregulation of activators (Benowitz and Carmichael 2010). For example, proteins that associate with CNS myelin, such as NogoA and myelin-associated glycoprotein (MAG), and scar tissue containing chondroitin and keratin sulfate proteoglycans at the ischemic injury site have been shown to inhibit axonal outgrowth (Benowitz and Carmichael 2010; Galtrey et al. 2007; Pizzorusso et al. 2002; Silver and Miller 2004). The effect of cell transplantation on such inhibitors is to date unknown. Stem cells may also act by activating the intrinsic growth potential of neurons. Expression of a number of growth-promoting molecules increases in the ischemic penumbra after stroke, and this gene expression pattern, not surprisingly, differs in the young versus aged brain (Carmichael et al. 2005; Li et al. 2010). In the young adult, many of the genes expressed in the sprouting neurons are linked to axonal sprouting or pathfinding during development (Li et al. 2010) including axonal guidance receptors such as neuropilin-1 (*Nrp1*) and L1 cell adhesion molecule (*L1cam*) and cytoskeletal modifying proteins such as stathmins (*Stmn3* and *Stmn4*). In contrast, aged sprouting neurons show a greater expression of immune-related genes, insulin-like growth factor-1 (*Igf1*) and bone morphogenetic proteins, and a distinct number of molecules that actually inhibit axonal growth such as the ephrin type-A receptor-4 (*Epha4*).

Transplanted cells could also enhance plasticity indirectly by modulating other processes that affect brain plasticity. For example, regrowth of blood vessels after stroke is important to support the survival and growth of neurons (and other brain cells) and undoubtedly will be necessary for brain plasticity. We and others have reported that hNPCs and hMSCs enhanced revascularization in the peri-infarct region after stroke (Chen et al. 2003; Hayashi et al. 2006; Horie et al. 2011; Onda et al. 2008). Moreover, by selectively immunodepleting hNPC-secreted VEGF using Avastin, an anti-human VEGF antibody that does not bind to rodent VEGF, we showed that VEGF secretion by the transplanted cells was essential for their enhancement of vascularization. Transplanted cells (hNPCs, MCSs, or human cord blood cells) can also modulate inflammation after stroke. They have been shown to not only downregulate inflammatory genes (Ohtaki et al. 2008) but also reduce the number of leukocytes in the brain (Horie et al. 2011; Lee et al. 2008; Vendrame et al. 2005). This immunomodulatory action of stem cells could influence brain plasticity not only by affecting neuronal survival but also by effects on synaptic plasticity, as many inflammatory cytokines (e.g., IL1, TNFα, IL6) can influence neural plasticity either in a beneficial or detrimental manner depending on their concentration (Yirmiya and Goshen 2011).

1.5.2 Integration into the Host Brain

The initial attraction of NPC cells for stroke therapy was their potential to become neurons and replace lost circuitry; however, evidence for this is limited. Transplanted NPCs in a rat model of global ischemia (Toda et al. 2001) and hNT neurons in a model of traumatic brain injury (Zhang et al. 2005) have been reported to express synaptic proteins. Electron microscopy studies revealed that human NPCs form synapses with host circuits after ischemia (Daadi et al. 2009; Horie et al. 2011; Ishibashi et al. 2004; Lee et al. 2008; Vendrame et al. 2005) and electrophysiological properties characteristic of functional neurons have also been shown for transplanted hNPCs (Buhnemann et al. 2006; Daadi et al. 2009). However, only very few synapses are seen, and recovery often occurs too early to be attributable to newly formed neuronal connections (Englund et al. 2002; Song et al. 2002), although such integration might be significant for recovery at later time points. Moreover, recovery is also reported with nonneuronal cells (e.g., MSCs) and when NPCs do not differentiate into mature neurons (Andres et al. 2011; Horie et al. 2011). Together, this implies that neuronal replacement is not necessary for cell-induced recovery, and the significance of the limited neuronal integration reported remains to be determined. NPCs also have the potential to become oligodendrocytes and astrocytes which could also integrate into the host brain and affect plasticity. Replacement of lost oligodendrocytes to remyelinate axons would be beneficial after stroke; remyelination by human NPCs was reported in spinal cord injury (Cummings et al. 2005); however, to date there are few reports of transplanted NPCs becoming oligodendrocytes in the ischemic brain (Daadi et al. 2008, 2009). Astrocytes play multiple roles in the brain including regulation of synapse formation and activity (Allen and Barres 2005), primarily through secretion of factors such as thrombospondins. Therefore, astrocytic integration could also be beneficial for stroke recovery.

1.6 Translational Implications of Stem Cell Therapy

Thrombolysis therapy using t-PA is the only means to improve functional recovery in the postischemic phase. This therapy, which aims to lyse the clot and restore blood flow, is limited in that it must be delivered within the first few hours after stroke. Many stroke patients are not eligible, however, because they arrive at hospitals well out of this time window. Cell transplantation therapies are not restricted to the same time limitations because they aim to enhance repair mechanisms. Most preclinical studies transplant within the first 3 days after stroke. Furthermore, studies have confirmed recovery within the subacute period (1 week post stroke) and chronic period (>3 weeks post stroke) (Andres et al. 2011; Borlongan et al. 1998; Chen et al. 2001b; Daadi et al. 2008; Horie et al. 2011; Pollock et al. 2006; Shen et al. 2007; Zhao et al. 2002). The timing of therapy should be considered as it may elicit differing clinical responses. To induce a neuroprotective response, acute delivery may be warranted whereas therapy during the first few weeks after stroke may promote repair mechanisms.

Proper patient selection will be crucial in determining the efficacy of stem cell therapeutics. Three factors should be considered in patient selection: patient demographics, lesion (location and size), and nature of stroke (hemorrhagic versus ischemic). Stroke typically affects the elderly population with significant comorbidities, such as hypertension, diabetes mellitus, and atherosclerosis. Aged rats have a less plastic gene expression profile after stroke (Li et al. 2010) and have higher astrocyte reactivity, increased macrophage recruitment, and delayed neuronal death after hemorrhagic stroke than younger rats (Wasserman et al. 2008). Therefore, further studies into the behavior of transplanted cells into young and mature brains need to be conducted.

Multiple means of cell delivery exist; the most common approaches are intracerebral, intravascular, and intracerebroventricular (Andres et al. 2008a; Bliss et al. 2007; Guzman et al. 2008b; Hicks and Jolkkonen 2009); however, the optimal means of delivery is still unknown. Intracerebral delivery may provide a greater number of transplanted cells than the other delivery routes. For larger stroke volumes, intravascular delivery may provide a wider distribution of cells to ischemic areas (Guzman et al. 2008b). Interestingly, many studies have shown significant recovery with a small number of cells (Guzman et al. 2008b; Hicks and Jolkkonen 2009; Li et al. 2002; Vendrame et al. 2004) or even no cells. In these cases, it is most likely that transplanted cells exert their effect through paracrine factors. Nevertheless, in the larger human brain, the question remains whether secreted factors must diffuse further or if they can act locally to alter surrounding circuits that alter remote remapping. Pending data from clinical trials should shed more light on these questions.

At a behavioral level, experiments in animals to favor the impaired limb by constraining the unimpaired limb have also resulted in increased axonal projections and synaptic densities and improved fine motor control (Maier et al. 2008). To design effective strategies for long-term recovery, therefore, paradigms to improve functional recovery after stroke should involve molecular aspects of synaptogenesis as well as behavior patterns through physical and occupational therapy.

Conclusions

Successful translation of stem cell therapy for cerebral ischemia requires not only answers to basic cell biology questions and knowledge of the molecular factors necessary for synaptogenesis and functional recovery but also identification of patient and donor cell factors as well as development of treatment protocols and outcome measurements (Abe et al. 2012). Patient factors include the appropriate age, stroke type, and patient comorbidities. Donor cell factors include identifying the optimal cell types, their safety profiles, and the safe and rapid protocol for ex vivo cell expansion. Treatment protocols include defining the timing for cell transplantation after the ischemic event, the best delivery route, and the most advantageous cell dose. Last, it is also important to choose the appropriate outcome measures, such as cell migration and integration tracking, brain function imaging, and behavioral functionality. Answering these questions successfully will require a great collaborative effort among scientists and clinicians. The data yielded thus far point to a promising future.

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