The emerging roles of transplanted radial glial cells in regenerating the central nervous system

Scientists conclude that a combination of treatments involving rehabilitation, drug delivery, surgery and cell transplantation are necessary to achieve significant progress in regenerating the injured central nervous system (CNS). The benefits of pluripotent stem cells in neurodegenerative disorders are well recognised (Thompson and Bjorklund, 2015) and cell culture methods have advanced to condition and enrich cells of therapeutic interest, thereby optimising their assimilation into diseased CNS regions. Radial glial cells are a unique cell type that act as both cell progenitors and scaffolds during development, orchestrating many brain and spinal cord formation events. In light of new developments elucidating their precursor and regenerative capacities, we will place a spotlight on the transplantation potentials of embryonic stem (ES) cell or cell line derived radial glia and endogenous radial glial cell populations in re-establishing neural connectivity and restoring cell populations following neurodegeneration.

Radial glial cells mediate neural connectivity during development: The proliferating CNS exists as a population of multipotent neural stem cells and progenitor cells arranged as polarized neuroepithelium which ultimately produces all neurons, glia and oligodendrocytes in the adult CNS. The earliest glial progenitors of the neuroepithelium are termed radial glial cells, a secondary class of neural progenitor which gives rise to the majority of brain neurons and astrocytes. Radial glia and their subtypes are found throughout the developing CNS and in neurogenic niches in the adult (Barry et al., 2014). They possess an apical - basal polarity which creates the architectural framework for the laminar patterning of migrating neurons (Rakic, 1972). This morphology also facilitates their interaction with axons along a number of axes providing boundaries, conduits and sorting structures in the brain and spinal cord (Norris and Kalil, 1991; Barry et al., 2013). These proliferative and boundary forming functions are especially significant when understanding the developmental defects affecting motor and sensory systems in vertebrates and present multiple therapeutic opportunities for radial glial cell transplantation into the degenerating or injured CNS. Moreover, the roles of radial glia in regenerative neuroscience in amphibians and fish are becoming more widely studied, where it appears they effectively safeguard the mature brain and spinal cord from permanent injury by restoring entire CNS compartments (Becker and Becker, 2015).

Transplantation of ES cells and cell line derived radial glial cells: A major obstacle to functional recovery after mammalian CNS degeneration is the near inability of damaged or dead neurons and glial cells to regenerate and restore synaptic connectivity. However, in recent decade a clearer understanding of the trophic factors, drug treatments, transplantation vectors and tissue scaffolds that promote the survival of neurons and glial cells have benefited the outcomes of cell replacement therapies. In particular, cell transplantation strategies aim to compensate for CNS defects using endogenous embryonic neurons, grafts of neural tissue or conditioned immortalized neural precursors that may or may not be genetically modified. Recently, increasing attention has been placed on the benefits of transplanting both differentiated and/or pluripotent ES cells. ES cells proliferate extensively (Thompson et al., 1998; Gage, 2000), yet their allogenic transplantation often results in tumour formation. This limitation has resulted in greater interest being placed on pre-differentiated ES cells that are less tumorigenic (Batista et al., 2014). For example, the transplantation of dopaminergic neurons in Parkinson’s disease (Shin et al., 2014; Han et al., 2015) and motor neurons in amyotrophic lateral sclerosis (Coatti et al., 2015) have yielded significant functional recovery in humans.

Generating primary radial glial cell cultures generally requires their co-culture with neurons or other brain extracts (Hunter and Hatten, 1995), making them difficult to study in isolation or to prepare pure populations for transplantation. However, cell line derived collections of radial glia and neurons, termed substrate adherent neural aggregates (SENAs), have proven to be an excellent source of neurally-fated cells and have been successfully transplanted into mouse models of Huntington’s disease (Dihne et al., 2006), Parkinson’s disease (Cui et al., 2010) and spinal cord injury (Cui et al., 2011), without tumour formation. The majority of cells in these aggregates initially show similarities to in vivo cortical radial glial cells, including nestin expression and a propensity to differentiate to neurons in culture (Dihne et al., 2006). However, their identity as true radial glial cells that recapitulate their in vivo counterparts is debatable. Notwithstanding the phenotypic consistency of SENAs, an isolated human neural stem cell line termed SD56, expressing vimentin, nestin and 3CB2 (markers of early appearing radial glia (Prada et al., 1995)) showed extensive migration without tumorigenesis around a striatal ischemic lesion in the rat, significantly improving the independent use of the stroke impaired forelimb (Gage, 1999).

Perhaps a more accurate paradigm of radial glial cell transplantation is the C6 glioma derived radial glia-like cell line C6-R. C6-R cells show a bipolar morphology in vitro and support neuronal migration, while expressing markers typical of in vivo radial glia including vimentin, nestin, glial fibrillary acidic protein (GFAP) and RC1 (Friedlander et al., 1998). These cells integrate well into the developing forebrain and are capable of adopting the typical radial glial apical - basal polarity without forming tumours over time. They also infiltrated into the adult forebrain and spinal chord white matter after implantation, where they supported the migration of co-implanted primary neurons in the healthy and lesioned cortex, corpus callosum and hippocampus (Hormigo et al., 2001a, b). While their potential in brain injury is clear, C6-R cells formed tumours when implanted into the contused spinal cord (Hasegawa and Grumet, 2003), rendering them impractical in spinal cord injury. However, a similar clone RG3.6 produces radial glial cells that express brain lipid binding protein (BLBP), glutamate aspartate transporter and vimentin, which are markers of mature radial glia in the brain and spinal cord, are bipolar and migrate extensively through the spinal cord white matter without forming tumours after transplantation (Hasegawa et al., 2005; Chang et al., 2009). When transplanted into the contused rat spinal cord, RG3.6 cells localized extensively above and below the injury epicentre and decreased the appearance of macrophages and chondroitin sulfate proteoglycans, thus limiting inflammation. Indeed, axon growth across the lesion was enhanced, leading to increased Basso Beattie Bresnahan scores of greater than two points within the first week following the injury (Hasegawa et al., 2005). Moreover, Chang et al. (2009) showed that transplanted RG3.6 cells upregulate various protective factors in the host including anti-apoptotic Hsp70 mRNA as well as the neurogenic and cell lineage factors Foxg1, Top2a, Nkx2.2 and Sox11, a factor critical for neurite growth and survival. Together these studies show
that both stem cell derived radial glia and aggregates of radial
glia and neurons repopulate and offer neurotrophic support at
various CNS lesion sites (Table 1).

The transplantation and manipulation of radial glia-like
cells such as pre-differentiated astrocytes is also effective in
improving outcomes following spinal cord injury by dorsolat-
eral funiculus transection of the adult rat cervical spinal cord
(Davies et al., 2011). In this study, human glial precursor cells
were differentiated into GFAP-expressing astrocytes using either
bone morphogenic protein (BMP) or ciliary neurotropic factor
(CNTF). Interestingly, cells differentiated with BMP expressed
brain-derived neurotrophic factor, connexin-43 and glutamate
transporter-1, which are proteins expressed in vivo in astrocytes.
Furthermore, rats transplanted with astrocytes differentiated
with BMP, but not undifferentiated precursors or CNDF-de-
derived astrocytes, exhibited elevated numbers of neurons at the
injury site and significantly improved functional recovery via
the grid walk test. Moreover, the recent rapid progression in our
understanding of cell reprogramming technologies has placed
induced pluripotent stem (iPS) cells as a viable alternative to ES
cells as the future of human cell therapy, thereby bypassing eth-
ical and transplantation rejection issues (Thompson and Bjork-
lund, 2015). Indeed, a recent report described the production of
human radial glial cells from human pluripotent stem cells that
performed similar lineage and patterning roles as in vivo after
ventricular transplantation (Duan et al., 2015). This represents
an exciting advance, yet at the time of writing the regenerative
potentials of iPS-derived radial glial cells in trauma conditions
such as spinal cord injury or stroke had not been reported.

Transplantation of endogenous radial glial cells: The most
widely understood use of endogenous radial glia in a clinical
context is in spinal cord injury, which likely underlies their in
vivo axon guidance capacities. Simply stated, as upper motor
neuron cell bodies are located in the motor cortex it is more
plausible to regenerate axons at the lesion site before they die.
Radial glial cells have been demonstrated to reappear in re-
sponse to injury, at least in the spinal cord. Shibuya et al. (2003)
reported 3CB2-expressing radial glial cells in both grey and
white matter regions near an adult thoracic spinal cord com-
pression lesion site 1 week after injury. Their processes became
radialized after 4 weeks, resembling their embryonic morphol-
y. In addition, Nomura et al. (2010) showed that endogenous
radial glial cells have the potential to differentiate and migrate
across the loci of a complete adult spinal cord transection site
promoting the movement of axons through a chitosan channel,
acting as a neural bridge. Likewise, White et al. (2010) demon-
strated that at 3 days post mid-thoracic contusion injury cells
expressing BLBP, but not GFAP, were present at the injury epicentre, some of which also expressed nestin. As these cells were no longer found at 7 days post injury, it is possible that the endogenous BLBP-expressing population differentiated into ma-
ture astrocytes between 3 and 7 days after contusion, and may be
manipulated in order to facilitate repair. While transplantation of
neurotrophic cells remains perhaps the most viable strategy for
many neurodegenerative diseases, improving the local microen-
vironment by introducing neurotrophic factors to the lesioned
areas also promotes synaptogenesis and native cell proliferation.
Interestingly, treatment of the contused spinal cord with trans-
forming growth factor-α (TGF-α) resulted in a shift in astrocyte
phenotype from hypertrophied and interdigitated to an elongat-
ed shape reminiscent of radial glia (White et al., 2011). Further-
more, in vitro experiments showed that dorsal root ganglion cells
co-cultured with TGF-α treated astrocytes exhibited neurite out-
growth capabilities similar to those cultured on laminin, while
fetal bovine serum-treated astrocytes had significantly shorter
neurites than those grown on laminin. In addition, fibroblast
growth factor (FGF) has demonstrated much therapeutic prom-
ise after injury in a variety of CNS regions. Goldshmit et al. (2014)
showed that FGF2 mediated a reduction in reactive astrocyte
invasion to the glial scar and an up-regulation of pro-regenerative
glial precursor cells, likely radial glia, in the grey and white mat-
ter, which accompanied significant motor recovery in the mouse.
FGF2 is currently being trialed in humans with cervical spinal
cord injury.

While difficult to purify and maintain in culture, endogenous
populations of early appearing neuronal stem/progenitor cells
(NSPCs) can be preserved in the short term and have been ho-
motopically transplanted into the neocortex, hippocampus, ol-
factory bulb and striatum, where they preserve their lineage fares
(Carletti et al., 2004). Furthermore, when heterotopically trans-
planted they respond to their local microenvironment by show-
ing site specific integration and differentiation (Gaillard et al.,
2003; Kallur et al., 2006). However, transplanted progenitors be-
come more fate restricted as development proceeds (Broek et al.,
1998; Pinaudeau et al., 2000). These temporal, lineage-restriction
characteristics of neural progenitors have been exploited as rat
cortical NSPCs were grown as neurospheres, expressing markers
typical of radial glia including BLBP and GFAP, and then trans-
planted to the lesioned adult cortex in a model of thrombotic
stroke, where they primarily differentiated to functional neurons
1 week after implantation and replaced lost cells (Prajeroa et al.,
2010). Furthermore, multipotent radial glial cells have been
transplanted in the contused spinal cord of adult rats following
isolation and co-culture with Schwann cells from the developing
spinal cord (Li et al., 2007) and forebrain (McMahon et al., 2010)
(Table 1). Both migration and integration was observed in these
cases and transplanted radial glia differentiated into neurons, oli-
godendrocytes and astrocytes (McMahon et al., 2010).

A further application for radial glial cells lies in retinal de-
generation as cell transplantation is rapidly becoming a viable
strategy for treating neuro-retinal diseases including retinitis
pigmentosa and age-related macular degeneration. A subtype of
radial glial cell, the Müller glial cell, has been assigned some
limited regenerative capacities in the mammalian retina follow-
ning neurotoxic insult (Ooto et al., 2004; Jayaram et al., 2014), an
attribute greatly magnified in fish (Yurco and Cameron, 2005).
Moreover, the survival and integration of transplanted retinal
stem cells into normal and diseased retinas in mice and pigs has
been reported and these cells expressed markers typical of radial
glial cells including RC2, Pax6 and Mash1 (Canola et al., 2007)
and nestin, vimentin and LewisX (Klassen et al., 2007). Many of
these cells migrated and differentiated into layer-specific retinal
cells, while Klassen et al. (2007) demonstrated their potential
to become subretinal photoreceptors cells. Moreover, purified
Müller glia express protein similarities with dopaminergic (DA)
neurons, including tyrosine hydroxylase, L-DOPA decarboxylase,
the nuclear receptor-related factor 1 and DA associated trans-
porter expression (Stutz et al., 2014). Their subsequent trans-
plantation into the striatum of hemi-Parkinson’s disease mice
resulted in increased DA and 3,4-dihydroxyphenylacetic acid
expression when compared to the contralateral brain, which
significantly enhanced motor functions (Stutz et al., 2014).

In a rapidly advancing field, biomaterial-based transplan-
tation platforms such as hydrogels and nanofibre scaffolds are
enhancing engraftment by allowing multiple cell matrices to
be implanted, thereby replacing both the cells lost due to
injury and the neurotrophic populations necessary to enrich
them and modulate immune responses at the injury site (Tam
et al., 2014). For example, we are currently growing radial glial
cell-rich cultures isolated from the embryonic spinal cord on
specialised biopolymers and aim to apply these to spinal cord
injury loci to recreate the supportive embryonic CNS microen-
vironment (unpublished).

Closing remarks: It is clear that intricate networks of radial
glial cells or their progeny form scaffolds that segregate/guide
growing axons, while contributing to both gliogenesis and neu-ogenesis during development. Although significant strides have
been made to elucidate their roles and regenerative potentials in
some injury paradigms, they seem as yet to be an untapped
resource to promote recovery in multiple neurological condi-
tions. Recent reports describing the ability of radial glial cells to
re-differentiate at injury loci, and offer neurotrophic support to
surviving cells in both amphibians and mammals, will ensure
that attention will continually be placed on radial glia and their
derivatives. By combining this research with technological de-
velopments in neural tissue engineering to support the growth
and transplantation of CNS progenitors, we are confident that
radial glial cells and in particular ES cell derivatives such as
RG3.6 cells will play significant roles in advancing cell replace-
ment and regeneration therapies.

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