Neural stem cells and cell replacement therapy: making the right cells

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ABSTRACT

The past few years have seen major advances in the field of NSC (neural stem cell) research with increasing emphasis towards its application in cell-replacement therapy for neurological disorders. However, the clinical application of NSCs will remain largely unfeasible until a comprehensive understanding of the cellular and molecular mechanisms of NSC fate specification is achieved. With this understanding will come an increased possibility to exploit the potential of stem cells in order to manufacture transplantable NSCs able to provide a safe and effective therapy for previously untreatable neurological disorders. Since the pathology of each of these disorders is determined by the loss or damage of a specific neural cell population, it may be necessary to generate a range of NSCs able to replace specific neurons or glia rather than generating a generic NSC population. Currently, a diverse range of strategies is being investigated with this goal in mind. In this review, we focus on the relationship between NSC specification and differentiation and discuss how this information may be used to direct NSCs towards a particular fate.

INTRODUCTION

Considerable media attention has brought stem cell research into the spotlight, not least because of the exciting possibilities of its application in cell replacement therapies for neurological disorders using NSCs (neural stem cells). Despite the obvious benefits promised by this field and some encouraging preliminary studies, in reality there still remains a gulf between theory and practice.

Leaving aside the highly contentious ethical debate surrounding the procurement and use of ES (embryonic stem) cells to generate NSCs, as opposed to adult sources of stem cells such as those obtained from bone marrow, we still lack a profound understanding of the basic biology of NSCs and how they can be manipulated to provide consistent and effective results in cell-replacement strategies. NSCs are defined by three main characteristics: they can self-renew, give rise to all of the major neural cell types, i.e. neurons, oligodendrocytes and astrocytes, and can repopulate a damaged region. The first of these characteristics ensures that a pool of NSCs is maintained at the same time as more restricted progeny are generated, and their ability to self-renew provides a convenient approach by which to expand the initial NSC population. Despite the use of primary fetal tissue as proof-of-principle in a number of clinical studies, including treatment of PD (Parkinson’s disease) patients [1,2], it is not a viable option for large-scale therapeutic application due to a lack of available tissue and ethical considerations. Therefore a readily expandable NSC population, possessing an innate ability to make the three major neural cell types and repair damage following injury or disease, is an obvious attraction.

Key words: cell replacement therapy, embryonic stem cell, neural stem cell, neurological disorder.
Abbreviations: AP, anterior-posterior; BMP, bone morphogenetic protein; CNS, central nervous system; DA, dopaminergic; DV, dorso-ventral; EB, embryoid body; EGF, epidermal growth factor; EGFP, enhanced green fluorescent protein; ES, embryonic stem; FGF2, fibroblast growth factor 2; MCAO, middle cerebral artery occlusion; MN, motor neuron; NPC, neural progenitor cell; NRP, neuronally-restricted progenitor cell; NSC, neural stem cell; PD, Parkinson’s disease; PI, positional identity; RA, retinoic acid; SGL, subgranular layer; Shh, sonic hedgehog; SVZ, sub-ventricular zone; TF, transcription factor.
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Nevertheless, NSCs for clinical applications must possess a large number of specific qualities. These cells must be readily available, expandable and karyotypically stable in vitro, be able to differentiate in a predictable and appropriate manner, be able to generate functional neural cells that integrate appropriately and consequently mediate functional repair. In short, NSCs must be predictable, efficient, effective and, of course, safe. It is thus profoundly important to choose the correct starting cell population.

CHOOSING A STARTING CELL POPULATION: MAKING NSCs

To date, many types of stem cells have been studied for their effectiveness in neural replacement strategies, including ES cells, NSCs and a range of non-NSCs. In this review, we will discuss the advantages and disadvantages of each (summarized in Table 1) as well as recent experimental evidence that highlights their potential use for clinical application.

ES cells

ES cells are pluripotent stem cells from which all tissues of the developing embryo are derived and this pluripotency, together with a capacity for unlimited self-renewal, makes them an attractive source of cells for NSC research. ES cells, first grown in vitro in the early 1980s [3,4], can readily be isolated from the ICM (inner cell mass) of a blastocyst, expanded in vitro with LIF (leukaemia inhibitory factor) and, subsequently, directed down a range of specific lineage pathways, usually via aggregates termed EBs (embryoid bodies), by exposure to appropriate exogenous signalling molecules (for a comprehensive review, see [5]). The generation of highly enriched populations of NSCs from ES cells in vitro has been the focus of concentrated efforts by many research groups. Commonly, following growth as EBs, cells are specified to adopt an NSC or an NPC (neural progenitor cell) fate by exposure to RA (retinoic acid) or by maintenance in chemically defined minimal medium in the presence of FGF2 (fibroblast growth factor 2), a potent mitogen. This latter process is selective for the survival of NPCs [6,7]. However, NSCs have also been derived from ES cells grown in co-culture with BMSCs (bone marrow-derived stromal cells) without the need for EB intermediates [8].

ES-derived NSCs or NPCs (more restricted multipotential progenitor cells) can then be manipulated further by cell culture conditions to generate different types of neurons and glia. This step is particularly pertinent in terms of generating NSCs for transplantation. The ability to simply generate neurons or glia is not sufficient; it is necessary for NSCs to be able to generate specific neurons or glia in a predictable and efficient manner. Several groups report that ES-derived NSCs commonly give rise to GABAergic and some glutamatergic neurons as well as astrocytes and occasionally oligodendrocytes [6,9], as do directly isolated NSCs [10,11]. However, commonly encountered neurodegenerative diseases including PD and ALS (amyotrophic lateral sclerosis) are characterized by a selective loss of DA (dopaminergic) and cholinergic neurons respectively, and thus replacement strategies must focus on NSCs able to generate these particular neurons in sufficient quantities. In attempts to manipulate the identity of ES cell-derived neurons or glia, a range of different cell culture conditions have been utilized with varying levels of success. For example, the addition of Shh (sonic hedgehog), FGF8 and AA (ascorbic acid) to culture medium at specific time points during in vitro neural specification of ES cells increases the number of DA and serotonergic neurons generated [12]. However, exposing these same cells to RA and Shh significantly increases the number of MNs (motor neurons) produced [13]. The significance of this manipulation of neuronal fate determination by extracellular signalling molecules will be discussed in more detail later.

Despite the obvious benefits of using ES cells to generate NSCs, there are associated caveats. Primarily, it is necessary to restrict these cells to a neural lineage [14]. With ES cells this requirement is acutely important since any remaining non-neural pluripotent stem cells could give rise to teratomas upon transplantation, and

<table>
<thead>
<tr>
<th>Stem cell type</th>
<th>Source</th>
<th>Pros</th>
<th>Cons</th>
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<tbody>
<tr>
<td>ES cell-derived NSCs</td>
<td>ICM of blastocyst</td>
<td>Pluripotent, unlimited proliferation and stable karyotype</td>
<td>Tumorigenicity, ethical considerations, purification (markers), and require neural specification</td>
</tr>
<tr>
<td>Embryonic NSCs</td>
<td>Embryonic CNS</td>
<td>Neural lineage-committed, non-tumorigenic, and regionally specific</td>
<td>Long-term maintenance and ethical considerations</td>
</tr>
<tr>
<td>Adult NSCs</td>
<td>SVZ and SGL of hippocampus</td>
<td>Neural lineage-committed</td>
<td>Long-term maintenance, restricted potential?, and limited availability</td>
</tr>
<tr>
<td>Non-NSCs</td>
<td>Bone marrow, skin, umbilical cord, blood etc.</td>
<td>No ethical considerations, plentiful/accessible supply, and generate autologous cells</td>
<td>Require neural specification, and restricted potential?</td>
</tr>
</tbody>
</table>
have been shown to do so in rodents [7]. This represents something of a dilemma as one of the problems in the field of NSC research remains the lack of a definitive NSC marker. Indeed, even the once widely accepted notion of markers that define differentiated neural cell types has been challenged [15]. A vast array of positive and negative selective markers are known that allow for the enrichment of NSCs from non-neural or neural cell populations, including LeX (Lewis X; [16]), the intermediate filament protein Nestin, PNA (peanut agglutinin), HSA (heat stable antigen; [17]) and the TFs (transcription factors) Musashi1 [18,19] and Sox1 [20]. However, none of these markers can be used to exclusively identify NSCs since many are found in more restricted NPCs or indeed in non-neural cell types. Although highly enriched populations of ES-cell derived NSCs can be purified by combining the use of these markers with careful manipulation of cell culture conditions, at present the possibility of residual pluripotent ES cells poses an inherent and unacceptable risk of tumour formation.

**NSCs**

An alternative to using ES cell-derived NSCs is to directly isolate NSCs. These cells have already undergone several steps of lineage commitment to produce stem cells destined to give rise to neurons, oligodendrocytes and astrocytes. Since NSCs are more lineage-restricted than ES cells, they represent less of a risk for tumour formation following transplantation. Indeed, from the many animal transplantation studies carried out, there is little evidence of their spontaneous tumorigenicity.

NSCs can be isolated from either the embryonic CNS (central nervous system) or from very restricted neurogenic regions now known to persist in two NSC niches in the adult brain: one in the SVZ (sub-ventricular zone) lining the lateral ventricles [21–23] and the other in the dentate gyrus of the hippocampus [24], although the multipotentiality of the latter remains a contentious issue [23].

In addition, more restricted multipotential NPCs with the capacity to give rise to both neuronal and glial progeny in vitro have been isolated from numerous adult brain regions in both rodents and humans [25–29]. It remains an important consideration whether or not it is necessary to begin with classically defined NSCs for cell-replacement therapy when more restricted NPCs, that already contain the desired regional specification, may be a more suitable alternative (for review, see [30]). Indeed, lineage-restricted NPCs have been shown to be able to integrate into the host system [31] and to elicit repair in animal models, including PD [32] and multiple sclerosis [33].

Similarly to ES-derived NSCs, NSCs directly isolated from the CNS also have the ability to self-renew and can give rise to neurons, oligodendrocytes and astrocytes. However, the kind of differentiated cell types that they generate vary depending upon the developmental stage and region from which they are isolated [11,34–36] and the in vitro conditions in which they are grown thereafter [11]. It has also become increasingly apparent that their capacity to yield a variety of specific neural cell types can be readily altered by manipulation of intrinsic and extrinsic cues, a point discussed in more detail below.

**Non-NSCs**

Apart from embryonic and adult sources of ES cells or NSCs, a large body of evidence has emerged over the last few years showing the capacity of non-NSCs, including bone marrow [37], skin [38] and umbilical cord [39,40] stem cells, to generate various types of neurons and glia. The obvious attraction of the use of these cells is that they are in ready supply and eliminate many of the ethical considerations that complicate our use of embryonic-derived cells. Furthermore, they raise the possibility of generating a large number of autologous NPCs for transplantation, thus eliminating concerns over host immune response to non-autologous grafted cells.

Although an in-depth discussion concerning the potential of non-NSCs to generate neural cells for transplantation is beyond the scope of this review, they represent a potentially exciting resource, which is evident from the ever-increasing number of reports revealing a heretofore unimagined plasticity [41].

Thus there are many pathways by which we can reach our goal: the generation of a population of neural cells for transplantation therapy, each with its own characteristic benefits and caveats (summarized in Table 1). In the next section, we outline our current understanding of the basic biology of NSCs, describing how intrinsic and extrinsic factors act to control their proliferation, specification and differentiation into specific types of neural cells. We discuss how this knowledge can be exploited by NSC researchers to manufacture NSCs with specific characteristics.

**SUPPLY AND DEMAND: ENOUGH NSCs TO GO AROUND**

As discussed earlier, ES cells are often used to generate NSCs because of their unlimited ability to proliferate in vitro. NSCs also retain the potential to self-renew, although their capacity to do so is finite, with rodent and human NSCs displaying different limitations in terms of long-term propagation [42,43]. Nevertheless, there have been two main ways in which NSCs have been maintained in vitro for expansion (Figure 1): (i) as free-floating clonally derived neurospheres, grown in the presence of the mitogens EGF (epidermal growth factor) and/or FGF2; or (ii) as adherent immortalized NSC lines, typically carrying an oncogene to facilitate continued
Figure 1  Two main ways in which NSCs are maintained in vitro

NSCs can be propagated in vitro either as neurospheres or as immortalized NSC lines. Neurospheres are generated from single NSCs by proliferation with the mitogens FGF2 and/or EGF and can generate all three major neural cell types: neurons, oligodendrocytes and astrocytes. However, being non-immortalized somatic cells, they have a finite lifespan in vitro. In contrast, NSC lines are generated by immortalization of NSCs using oncogenes (such as v-myc) or overexpression of the catalytic subunit of telomerase (TERT). This allows their sustained growth in vitro. These cells can also generate all three major neural cell types but, of course, these cells are now genetically modified, a possible problem for use in human transplantation.

proliferation, again grown in the presence of FGF2 (and/or EGF). One of the reasons for the senescence of NSCs maintained long-term in vitro is believed to be telomere shortening [42]. Previous studies have shown that NSC immortalization using the oncogene v-myc causes increased telomerase activity, thus maintaining a stable karyotype and permitting extensive perpetuation of human NSC lines in vitro [44]. Similarly, FGF2 has been shown to mediate a dose-dependent increase in telomerase activity in NPCs isolated from the embryonic mouse cortex [45]. Alternatively, it is possible to immortalize NPCs by ectopic expression of TERT, the catalytic subunit of human telomerase, shown to sustain human NPC expansion in vitro [46].

In terms of their ability to promote functional repair in the damaged brain, although a number of rodent studies using immortalized NSC lines show evidence of functional repair [47–49], less have been reported using neurosphere-derived cells [50]. In these types of studies, the migrational properties, survival and capacity of NSC populations to differentiate remain unpredictable and their mode of repair is poorly understood.

The ultimate goal of NSC research for replacement therapy is to generate large numbers of NSCs or NPCs that will efficiently undergo site-specific differentiation before correctly integrating into the host environment to mediate functional recovery. How can this goal be achieved? Can NSCs meet all of the requirements and how will they respond to the host environment? During embryonic development, the diverse cell types in the brain are generated in an exquisitely precise spatio-temporal order under the control of both intrinsic and extrinsic factors. Can we imitate the way in which neural cell fate is determined in the developing embryo to produce ‘designer’ NSCs, programmed to adopt particular cell fates?

RIGHT CELL, RIGHT PLACE: LESSONS FROM DEVELOPMENT

Patterning along the neuroaxis during embryogenesis is initiated by gradients of specific diffusible molecules secreted from signalling centres that are responsible for the induction of PI (positional identity). These factors interact both synergistically and repressively with one another in a concentration-dependent manner to switch on cascades of downstream TFs that define specific populations of NPCs in order to generate the broad range of cells that constitute the adult brain (for in depth reviews, see [51–57], and research articles, see [11,58]). This is illustrated in Figure 2, with two opposing gradients of diffusible signalling factors initiating a concentration-dependent expression of different TFs along the DV (dorso-ventral) axis [this is equally applicable to patterning of the AP (anterior-posterior) axis]. The repressive or inductive interactions between TFs refine further the boundaries between each expression domain. Each progenitor domain then generates particular differentiated cell types (Figure 2, cell types 1–5). In this way, specific regions composed of specific types of neurons and glia develop along the DV and AP axes of the CNS. Despite the distinctive characteristics of each adult brain region, the same underlying mechanism of generating cellular diversity is used along the extent of the developing neuroaxis, simply
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Figure 2  Simplified representation of the mechanism of DV patterning of the neuroaxis

Two opposing gradients of diffusible signalling molecules, A (black) and B (white), are expressed in the developing neural tube. This establishes broad overlapping regions of specific concentrations of A and B, which can interact with one another (black/grey/white bar). The relative concentrations of these signalling molecules regulate the expression of specific sets of TFs along the DV axis which, in turn, can activate or repress one another (NB this equally applies to the AP axis). This leads to the formation of distinct and discreet regions of TF expression (solid coloured rectangles from black to pale grey), which specify the fate of subsequent progeny from each region, producing different types of neural cells (illustrated as cell types 1–5). In this way, the diverse range of cell types required to generate particular regions of the brain is obtained. Similar mechanisms establish DV and AP patterning along the entire length of the developing neural tube, each with its own combination of diffusible signalling molecules and region-specific TF expression.

by using different sets of diffusible signalling molecules that regulate the expression of different downstream TFs.

NPCs isolated from different progenitor zones within the embryonic brain thus contain an intrinsic specification, conferred by their TF expression profile. When NPCs are grown in vitro they are known to retain their PI and to generate neurons or glia appropriate for their region and developmental stage of origin ([11,50, and A. Bithell and B. P. Williams, unpublished work]).

MAKING THE RIGHT TYPE OF NEURON

To illustrate the point above relating PI to subsequent cell fate, NPCs from the developing midbrain express the orphan nuclear receptor TF, Nurr1. Nurr1 is also highly expressed in DA neurons of the midbrain, which are lost in PD, and it has been shown to instruct midbrain NPCs to adopt DA neuronal fates. Numerous studies report the ability of Nurr1 overexpression in CNS precursors [59] or ES cells [60] to increase the yield of DA neuronal progeny and even a subsequent ability to promote functional repair following transplantation in rodent models of PD [60].

Using this knowledge, it seems logical that isolating NSCs from the appropriate region of the embryonic brain or engineering NPCs with a specific PI could provide a convenient source of pre-programmed progenitor cells that display the requisite gene expression. It is therefore important to determine the possibilities and limitations of using PI to predict neural cell fate determination both in vitro and following transplantation.

MAINTAINING PI IN VITRO

There are numerous caveats with using NSCs or more restricted progenitors (NPCs) from particular regions of the CNS to obtain predictable differentiated cell types. Firstly, although NSCs/NPCs express regional-specific TFs when isolated from the embryo, their subsequent propagation and maintenance in vitro reveals that this does not remain the case; the necessity for exogenous mitogens for continued NSC proliferation, particularly FGF2 (as discussed above), has been reported in numerous studies to alter their intrinsic positional specification [11,61,62]. A good example of this is provided by a recent study by Hack and co-workers [11] involving the generation of neurospheres from the dorsal or ventral embryonic telencephalon. In vivo, as dorsal telencephalic NPCs express dorsal-specific TFs, including Pax6, Emx2 and Ngn1 and Ngn2, and give rise to glutamatergic neurons and later to astrocytes [63–66]. Conversely, ventral NPCs express ventral-specific TFs including Gsh2, Mash1, Dlx2 and Olig2, giving rise first to GABAergic and cholinergic neurons, and then to oligodendrocytes later in development [67–71]. Hack and co-workers [11] reported that, although primary NPCs isolated from these regions express the appropriate TFs in vitro and generate the expected types of neurons or glia, propagation of these cells as neurospheres in the presence of FGF2 leads to a dysregulation of regional TF expression. The outcome of this is that dorsal spheres ectopically express ventral factors, including Olig2, and produce differentiated cell types with a ventral phenotype, often GABAergic neurons, while down-regulating dorsal-specific TFs. Similarly, a second study [10], comparing human and mouse embryonic NPCs, has shown an exclusive bias for the generation of GABAergic neurons above other types of neuron following repeated passaging with FGF2 and EGF.

The requirement for FGF2 (or indeed EGF) may therefore be a barrier to maintaining the positional specification of isolated NSCs. In particular, neurospheres have increasingly been shown to alter their gene expression profiles following long-term passage in vitro, although the extent to which they do this is still a matter of contention, with conflicting findings.
Comparison of findings from neurosphere-based experiments to analyse retention of NSC regional specificity

Table 2  Comparison of findings from neurosphere-based experiments to analyse retention of NSC regional specificity

<table>
<thead>
<tr>
<th>Authors</th>
<th>Age of isolation</th>
<th>Brain region</th>
<th>Expansion of neurospheres</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parmar et al. [35]</td>
<td>E13.5</td>
<td>CTX, LGE and MGE</td>
<td>EGF and FGF2 for six passages</td>
<td>Cells largely retain regional specification (ventral-specific TFs Dlx, Pbx, Mies and Nkx2.1 predominantly in LGE and not CTX).</td>
</tr>
<tr>
<td>Hack et al. [11]</td>
<td>E14</td>
<td>CTX</td>
<td>EGF and FGF2 for four passages</td>
<td>Cells quickly obtain similar TF expression profiles. Most dorsal-specific TFs are down-regulated, and ventral-specific Olig1 and Olig2 are up-regulated.</td>
</tr>
<tr>
<td>Hitoshi et al. [72]</td>
<td>E14.5</td>
<td>CTX, GE, MB/rHB, and cHB</td>
<td>FGF2 for two passages, then EGF and FGF2 for 7 days</td>
<td>Cells largely retain regional identity appropriate to DV and AP position. Only GE cells display appropriate migratory response to local cues in GE slice cultures.</td>
</tr>
<tr>
<td>Gabay et al. [61]</td>
<td>E13.5</td>
<td>SC</td>
<td>EGF and FGF2, non-passaged neurospheres</td>
<td>FGF2 up-regulated the expression of Olig2 in Olig2-derived neurospheres permitting oligodendrocyte differentiation. Olig2 down-regulation permits their differentiation into astrocytes.</td>
</tr>
<tr>
<td>Santa-Olalla et al. [62]</td>
<td>1-day intervals from E11.5–E15.5</td>
<td>CTX, GE, Dienc, dM, vM, Mt, My and SC</td>
<td>EGF or FGF2 for 7 days</td>
<td>Cells from all regions display ectopic or reduced expression of AP and DV markers. Pax6 is up-regulated in all cells, irrespective of origin.</td>
</tr>
</tbody>
</table>

either supporting [35,72] or refuting [11,61,62] sustained regional specification. This is despite the similarities between starting neural cell populations and culture conditions in these studies, the main findings of which are summarized in Table 2. To fully understand the influence of FGF2 (and EGF), future work must first aim to reconcile these disparate observations.

Other NSC handling methods also have a dramatic effect on the repertoire of progeny obtained; these include cell density and passage number. Furthermore, the age from which NSCs are isolated affects their intrinsic capacity to differentiate into particular cell types; a mechanism established in vivo to generate first one cell type followed by another in a temporal manner from a common progenitor. It is therefore essential that we carefully consider the influence in vitro manipulation has on PI and cell fate when generating cells for transplantation therapy.

**INTRINSIC AND EXTRINSIC FACTORS ABLE TO ‘PROGRAMME’ NSCs**

We already know that regions of the developing brain are patterned by gradients of different secreted and diffusible signalling molecules and that the TFs regulated downstream of these molecules, responsible for conferring PI, then specify NPC fate. As discussed above, it has been suggested recently that mitogens utilized in vitro, particularly FGF2, might be responsible for altering the expression of PI genes in NSCs, thus affecting their capacity to generate particular cell types. Although this may be an impediment to using these in vitro systems to accurately investigate the basic biology of NSC lineage determination in vivo, it does suggest a means by which the intrinsic patterning of an NSC can be overridden by exposing these cells to specific diffusible signalling molecules.

Using telencephalic development as an example, glutamatergic neurons of the developing cortex are generated from the progenitor zones of the cortex. However, most, if not all, GABAergic neurons of the cortex are generated in the ventral telencephalon and must follow a tangential pathway of migration to reach their final destination in the cortex [73,74]. It is now known that the reason behind this is that the development of GABAergic neurons requires expression of TFs that are specifically expressed in the ventral telencephalon.

The ventralization of the telencephalon requires Shh. Conversely, members of the WNT (Wingless-related) and BMP (bone morphogenetic protein) families are involved in its dorsalization ([75]; for review, see [57]). A recent study by Gulacsi and Lillien [58] showed that NPCs from the dorsal telencephalon, which usually give rise to glutamatergic neurons, could be made to differentiate into GABAergic interneurons by addition of exogenous Shh. This ability to alter the fate of dorsal NPCs is mediated by the antagonistic effect of Shh on BMP4 and, indeed, simply blocking the action of BMP4 in the absence of Shh was sufficient to induce GABAergic neuronal differentiation. Similarly, the levels of BMP expression can alter the progeny of a Shh-responsive ventral progenitor cell from GABAergic neurons to oligodendrocytes [71].
Using exogenous signalling molecules, it is thus possible to modulate the fate of NPCs in vitro. By investigating the effect of different molecules on the intrinsic gene expression profile and concomitant cell fate of an NSC or NPC, we may be able to generate neural cells that differentiate in a specific manner. That we can alter the intrinsic PI of an NSC or NPC and, hence, its fate also raises the possibility that a truly specific progenitor does not exist. Indeed, the specificity of NSCs or NPCs in vivo may merely reflect their environment.

FUNCTIONAL REPAIR: MAKING APPROPRIATE CONNECTIONS

Although it is critical for NSC replacement to generate the appropriate types of neurons or glia once grafted, this is of little consequence if the cells cannot integrate into the host system to mediate functional recovery. Currently, although many in vitro studies show that NSCs generate functional neurons (for example, neurons that display action potentials) and glia, there remains relatively sparse corresponding in vivo evidence.

A recent study by Wernig et al. [7] highlights the ability of transplanted NSCs to functionally integrate into a recipient host. In this study, NSCs generated in vitro from ES cells derived from a tau:EGFP (enhanced green fluorescent protein) knock-in mouse were transplanted intraventricularly into E16.5 mouse embryos; all subsequent neuronal progeny could thus be identified by EGFP expression. Transplanted EGFP-expressing NSCs not only migrated to diverse regions of the brain but also integrated functionally: they formed synapses with host cells, displayed complex morphologies and illustrated active and passive membrane properties characteristic of functionally integrated cells. However, the neurons displayed a range of neurotransmitter subtypes with no particular regional specificity. Furthermore, analysis of TF expression revealed that integrated cells showed a mixture of appropriate and ectopic TF expression. Although this evidence reveals that appropriate regional PI is not a prerequisite for functional integration and that the NSCs are able to generate a diverse range of neurons, it remains to be shown that the connections made between host and transplanted neurons are appropriate and could promote repair. As alluded to previously [11], it is also evident that NSCs are not homogeneous and represent a population at different stages of commitment and therefore with different capacities to respond to environmental cues.

A more successful approach has recently been reported using ES cells engineered to express a specific TF. In this study [76], ES cells were taken from a transgenic mouse expressing EGFP under control of Hb9, an MN-specific TF, and grown in vitro in conditions to induce MN differentiation. These MNs subsequently expressed appropriate receptors and neurotransmitter phenotype, and when co-cultured with muscle cells were able to display appropriate action potentials and functional synapses. Although conducted in vitro, this work highlights the question of whether or not it is more beneficial to transplant cells that have already been specified in vitro to generate the cell types required. Transplantation of less restricted NPCs (or NSCs) puts greater emphasis on the requirement of appropriate local cues for correct differentiation, migration and integration. This is made evident in a study by Han et al. [31], who reported that NRPs (neuronally restricted progenitor cells) transplanted into the spinal cord can generate mature neurons, whereas NSCs fail to do so, even though they can generate neurons if transplanted into the dentate gyrus [77]. The authors [31] suggest that this difference is due to the fact that NRPs do not require neurogenic signals to generate neurons, having already undergone specification to a neuronal lineage. In contrast, unspecified NSCs require neurogenic signals not present or that are inhibited in the normal adult spinal cord. Instead, NSCs give rise to glial cells, particularly astrocytes, in response to the gliogenic signals present in the adult spinal cord.

FUNCTIONAL REPAIR WITHOUT REPLACEMENT

Although the goal of NSC transplantation discussed above is achieved by cell replacement, it is important to briefly discuss the alternatives that have emerged over the past few years.

An interesting and perhaps unexpected finding came from a study by Veizovic et al. [48], which involved transplantation of a conditionally immortalized mouse NSC line, MHP36, into a rat model of ischaemic injury. In this model, a lesion is created in one side of the brain by MCAO (middle cerebral artery occlusion) and ischaemic damage can be observed by an asymmetry in the rat’s abilities to remove sticky paper placed on to its paw. Following transplantation of MHP36 cells, lesioned rats displayed responses similar to non-lesioned rats, whereas lesioned sham-grafted animals displayed the asymmetrical bias. The interesting aspect of this study came from the discovery that the MCAO-induced lesion in transplanted animals was significantly reduced, but this reduction could not be entirely attributed to the transplanted cells replacing lost host cells. Instead, the authors suggest that the transplanted NSCs acted to preserve and rescue host cells.

How might NSCs be able to promote repair without replacement? It is likely that at least one of the ways in which grafted NSCs can do this is by secreting factors that
are conducive to host cell survival, such as neurotrophic factors. Furthermore, as discussed earlier, there are regions of the adult brain that contain NSCs and continue to produce neurons in the adult [the SVZ and the SGL (subgranular layer) of the hippocampus], as well as other regions from which NPCCs have been isolated. Transplanted NSCs may be able to stimulate de novo production of neural cells from these remaining progenitors in the host brain. 

This latter point has fuelled a large amount of research into the innate ability of the adult brain to self-repair. Although NPCCs and NSCs are known to reside in the adult brain, with the exception of the SVZ and SGL, these cells do not normally make new neural cell types in the adult. We presume that signals able to influence the fate of these progenitors are simply not expressed in the adult. Providing these necessary signals to stimulate the brain to manufacture new cells from existing progenitors offers a conceivable alternative to cell replacement.

FUTURE OF NSC RESEARCH

Although major advances have been made in our understanding of NSC biology, there are still enormous hurdles before NSC transplantation can ever become commonplace. Specific questions that we need answers for are: which cells should we use as a starting point? How far should we restrict an NSC to a specific cell fate before transplantation? Can we programme cells in vitro to generate predictable cell types in vivo and how important is regional specific gene expression for site-specific differentiation and appropriate functional integration? We must also not forget that, although human and rodent NSCs and NPCCs share many characteristics, evidence is plentiful that there are also many differences and a direct comparison cannot always be made.

Although many questions still remain unanswered, the wealth of rapidly emerging evidence in this field is encouraging. Should we be able to gain the necessary depth of understanding of NSC specification, it is not inconceivable that routine NSC therapy will be a reality in the future. In the meantime, we continue to learn more about the elegant ways in which the complexity of the adult brain is established from NPCs in the developing embryo.

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