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Review

Moving Stem Cells to the Clinic: Potential and Limitations for Brain Repair

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Stem cell-based therapies hold considerable promise for many currently devastating neurological disorders. Substantial progress has been made in the derivation of disease-relevant human donor cell populations. Behavioral data in relevant animal models of disease have demonstrated therapeutic efficacy for several cell-based approaches. Consequently, cGMP grade cell products are currently being developed for first in human clinical trials in select disorders. Despite the therapeutic promise, the presumed mechanism of action of donor cell populations often remains insufficiently validated. It depends greatly on the properties of the transplanted cell type and the underlying host pathology. Several new technologies have become available to probe mechanisms of action in real time and to manipulate in vivo cell function and integration to enhance therapeutic efficacy. Results from such studies generate crucial insight into the nature of brain repair that can be achieved today and push the boundaries of what may be possible in the future.

Introduction

Most degenerative, vascular, inflammatory, or traumatic neurological diseases lead to an irreversible demise of brain tissue at some point during the disease course, which commonly goes along with deteriorating physical or intellectual function. Apart from the limited potential for endogenous regeneration in the human brain, which can be enhanced by rehabilitative training, treatment of such disorders is largely symptomatic. Symptomatic treatment usually involves the modulation of neurotransmitter systems and, for a growing number of pathologies, deep brain stimulation. However, symptomatic therapies often achieve only transient and partial efficacy and remain ineffective for several disorders. The identification of disease modifying drugs is highly desirable and is being pursued by the pharmaceutical industry (AlDakheel et al., 2014; Caraci et al., 2013). However, for most neurological disorders such drugs have not yet reached the clinic with a few notable exceptions such as in the case of relapsing-remitting multiple sclerosis (Smith et al., 2010). Given this medical dilemma, which represents a major socio-economic burden for many aging societies, experimental stem cell therapies hold considerable promise for brain repair. Research activities in neural transplantation have steadily increased since the initial reports of fetal tissue grafting in experimental models of Parkinson's disease (PD) (Brundin et al., 1986; Dunnett et al., 1981) followed by early clinical trials in PD (Lindvall et al., 1989, 1990) and Huntington's disease (HD) patients (Bachoud-Lévi et al., 2000; Reuter et al., 2008). Here we review the progress and remaining challenges toward the generation of unlimited numbers of defined human donor cell populations with therapeutic relevance to CNS disorders. We continue to describe the benefits and caveats that go along with the use of these cell populations in preclinical studies and impending clinical trials. We highlight the use of emerging technologies, which are geared toward increasing therapeutic efficacy, mapping connectivity, or interrogating mechanisms and therapeutic rationale. The potential for endogenous regeneration has been reviewed elsewhere recently (Dimyan and Cohen, 2011; Saha et al., 2012) and is not discussed here except for selective examples that highlight specific mechanisms or experimental approaches. We acknowledge that many therapeutic principles have been first described using rodent primary or mouse embryonic stem cell (ESC)-derived donor cells. However, since this review focuses on the prospect for human therapy, studies employing non-human cells are only mentioned if they demonstrate a unique principle not yet recapitulated with human cells.

I. Generation of Neural Cell Types from Various Sources

Primary Cells

While a number of non-neural tissue sources such as adrenal medulla autografts in PD have been used in the past (Backlund et al., 1985; Madrazo et al., 1987), the main era of neurotransplantation started with the use of fetal brain tissue as human donor tissue source. Early preclinical studies employed rodent (Dunnett et al., 1981), and later human (Brundin et al., 1986), cells derived from the fetal ventral midbrain in experimental models of PD. These studies provided strong evidence for the survival and therapeutic efficacy of mesencephalic dopaminergic grafts. As a consequence, the first clinical transplantation trials utilizing these cells in PD patients ensued swiftly. Despite promising data indicating motor recovery in the initial open label studies (Lindvall et al., 1989, 1990; Wenning et al., 1997), the two double-blind, placebo-controlled trials in PD patients (Freed et al., 2001; Olanow et al., 2003) failed to reach their primary endpoints. These studies also revealed graft-induced dyskinesias as a troubling side effect, which may be caused by contaminating serotonergic neurons in the donor cell population (Politis et al., 2010) though other factors may contribute as well. In some cases, however, grafts have been shown to survive for more than 15

years, to grow axonal projections, and to secrete dopamine as shown by [18F]Fluorodopa positron emission tomography (PET) scans and postmortem analysis. Also, subgroup analysis revealed significant effects in patients receiving a transplant under the age of 60 and patients followed for longer periods of time (Ma et al., 2010). Therefore, multiple factors such as patient selection (age, disease severity, L-Dopa responsiveness) and trial design (target site, immunosuppression, endpoints) as well as issues related to the donor cell populations are likely critical factors for success (Barker et al., 2013a). The donor cell populations in those studies varied with respect to gestational age, number of donors, pre-transplantation derivation, and storage as well as dopamine neuron content. However, beyond confounding biological and technical factors, obtaining up to seven donors simultaneously for transplantation of a single PD patient represents a serious logistical challenge and raises ethical concerns for a disease affecting millions of patients. In a related approach, HD patients have been grafted with fetal striatal tissue in small open label studies as well as in ongoing multicenter efforts involving several hundred patients. Long-term follow-up in at least a subset of those studies suggests benefits on motor and cognitive function for a period of several years following transplantation (Bachoud-Lévi et al., 2000, 2006; Reuter et al., 2008). Functional benefits may correlate with the extent of graft survival as determined by PET imaging (Bachoud-Lévi et al., 2006; Barker et al., 2013b; Reuter et al., 2008), though many other factors likely contribute.

Primary Neural Stem Cells

Given the concerns related to the use of primary fetal cells, a major focus in the field has been the generation of scalable cell populations that can be developed into standardized and quality controlled products for future therapeutic use. The ability to isolate neural stem cells (NSCs) in vitro and evidence of lifelong neurogenesis in some regions of the mammalian brain, reviewed in Gage and Temple (2013), argue for NSCs as one potential cell source. In vitro expanded rat fetal midbrain precursors have been shown to recover motor deficits in Parkinsonian animals (Studer et al., 1998), but the extent of cell expansion is limited using this approach and has never been developed into robust technology for use with human cells (Cave et al., 2014; Sánchez-Pernaute et al., 2001). Human NSCs obtained from the fetal telencephalon were shown to be expandable in vitro after immortalization (Flax et al., 1998), as neurospheres (Caldwell et al., 2001; Uchida et al., 2000) or in adherent monolayer cultures (Sun et al., 2008) in the presence of epidermal and/or fibroblast growth factors (EGF, FGF). Similarly, multipotent neural progenitor cells were expanded from several regions of the adult human brain (Nunes et al., 2003; Walton et al., 2006). These populations were shown to survive transplantation into immuno-compromised rodents and differentiate mostly into neurons and astrocytes in vivo. However, fate specification and therapeutic potential of the resulting neurons appear to be restricted, and differentiation into defined, authentic neuronal subtypes such as striatal projection neurons or midbrain dopamine neurons has never been shown. In fact, continuous exposure to mitogens such as EGF and FGF in the absence of additional patterning factors seems to interfere with the fate potential of the region of origin (Jain et al., 2003). In addition, the progressive switch from neurogenesis to gliogenesis occurring in many of the primary expanded populations (Naka-Kaneda et al., 2014; Patterson et al., 2014) can further complicate the use of NSCs in regenerative therapies. Nevertheless, despite their limited differentiation potential, NSCs are currently being tested in a variety of preclinical and clinical applications (see Primary NSCs).

Neural Cells Derived from Pluripotent Stem Cells

The isolation of human embryonic stem cells (hESCs) (Thomson et al., 1998) and subsequently induced pluripotent stem cells (iPSCs) (Takahashi et al., 2007) offered a new strategy to potentially generate any cell type in unlimited numbers. The generation of differentiated neural cell types from hESCs (Reubinoff et al., 2001; Zhang et al., 2001) was followed by the derivation of several developmentally distinct NSC populations (Elkabetz et al., 2008; Koch et al., 2009) reviewed in Conti and Cattaneo (2010). These pluripotent stem cell (PSC)-derived NSC populations displayed properties comparable to primary NSC sources with respect to expandability and differentiation potential with improved but still limited ability to control neuron subtype specific fates. In parallel, several strategies were developed to generate region specific neurons from human PSCs (hPSCs) without relying on a NSC intermediate. The strategy of directed differentiation has gradually evolved toward defined culture systems. Initial protocols commonly made use of stromal feeder cells or embryoid body (EB) cultures to enhance neural induction and further neuronal differentiation (Roy et al., 2006; Vazin et al., 2008). The increasing understanding of processes that regulate early mammalian CNS development and the availability of recombinant morphogens and growth factors led to a transition toward more defined differentiation protocols (Pera et al., 2004). An additional level of sophistication and efficacy was achieved with the use of small molecules, which activate or inhibit key developmental pathways such as Wnt, Shh, Activin/Nodal, BMP, TGF signaling (Smith et al., 2008). Harnessing these developments, a rapid, highly efficient, and surprisingly facile protocol has been devised, which generates PAX6+ primitive neuroectoderm within 10 days by inhibition of transforming growth factor β (TGF-β) and BMP signaling, also known as dual SMAD inhibition (dSMADi) (Chambers et al., 2009). The most attractive feature of dSMADi, however, is its malleability and modularity. Using the timed addition of one or several other patterning factors, a multitude of disease-relevant human neural cell populations have been derived in a systematic manner and with unprecedented efficiency and purity.

Projection Neurons

Excitatory glutamatergic projection neurons represent the main building blocks of the human telencephalon (Lui et al., 2011) and are affected in a large number of neurological diseases with different etiology. Interestingly, most neural differentiation protocols, whether feeder or EB based (Elkabetz et al., 2008; Koch et al., 2009; Li et al., 2005) or based on any form of SMAD inhibition (Chambers et al., 2009; Espuny-Camacho et al., 2013), pass through a dorsal telencephalic PAX6/OTX2 double positive intermediate. The acquisition of telencephalic fates is believed to represent a ground state of neuroepithelial cells during hPSC differentiation in the absence of additional patterning factors. Small molecule inhibition of canonical Wnt signaling was shown to further enhance telencephalic (FOXG1)