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Review

Reconstruction of brain circuitry by neural transplants generated from pluripotent stem cells

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ABSTRACT

Pluripotent stem cells (embryonic stem cells, ESCs, and induced pluripotent stem cells, iPSCs) have the capacity to generate neural progenitors that are intrinsically patterned to undergo differentiation into specific neuronal subtypes and express *in vivo* properties that match the ones formed during normal embryonic development. Remarkable progress has been made in this field during recent years thanks to the development of more refined protocols for the generation of transplantable neuronal progenitors from pluripotent stem cells, and the access to new tools for tracing of neuronal connectivity and assessment of integration and function of grafted neurons. Recent studies in brains of neonatal mice or rats, as well as in rodent models of brain or spinal cord damage, have shown that ESC- or iPSC-derived neural progenitors can be made to survive and differentiate after transplantation, and that they possess a remarkable capacity to extend axons over long distances and become functionally integrated into host neural circuitry. Here, we summarize these recent developments in the perspective of earlier studies using intracerebral and intraspinal transplants of primary neurons derived from fetal brain, with special focus on the ability of human ESC- and iPSC-derived progenitors to reconstruct damaged neural circuitry in cortex, hippocampus, the nigrostriatal system and the spinal cord, and we discuss the intrinsic and extrinsic factors that determine the growth properties of the grafted neurons and their capacity to establish target-specific long-distance axonal connections in the damaged host brain.

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Introduction

The use of cell transplants for brain repair is based on the idea that grafted neurons can become integrated into damaged brain circuitry, replace lost neurons, and reconstruct some critical aspects of damaged neuronal connectivity. The idea that immature neurons or neural precursors can be used to re-establish lost or damaged axonal

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connectivity and boost the regenerative capacity of the central nervous system (CNS) has been pursued experimentally in rodents since the 1970s, but it is only recently that serious attempts have been made to use cells derived from embryonic stem cells (ESCs) or induced pluripotent cells (iPSCs) with this goal in mind. Significant improvements in protocols for directed differentiation of human pluripotent stem cells and innovative approaches to study connectivity of transplanted neurons have pushed the field forward considerably. In particular, recent studies have provided new interesting insights into the capacity of ESC- and iPSC-derived neurons to establish new functional connections in brain and spinal cord in various lesion and disease models.

In this review we summarize these recent developments in the perspective of earlier studies using intracerebral and intraspinal transplants of primary neurons derived from fetal brain, and discuss the intrinsic and extrinsic factors that determine the growth properties of the grafted neurons and their capacity to establish target-specific long-distance axonal connections in the damaged host brain. We will limit ourselves to studies using cells derived from pluripotent stem cells – ESCs and iPSCs – and focus on four transplantation targets where some of the most interesting studies have been performed, *i.e.*, cortex, hippocampus, nigro-striatal system, and spinal cord.

Studies of graft–host connectivity derived from grafted primary neurons using classical anatomical and immunohistochemical methods

The early studies on anatomical integration of neural transplants relied on histochemical or immunohistochemical methods for selective visualization of specific neuronal systems, defined either by their transmitter content, or on the use of species-specific antibodies that allowed visualization of, *e.g.*, mouse, pig or human neurons and their projections in the rat brain. These tools were combined with classic anterograde and retrograde tracers injected into the graft tissue or into selected targets in the host brain. The transmitter-specific methods allowed visualization of grafted dopaminergic, noradrenergic and serotonergic neurons in their entirety, including the totality of their axonal projections in the host brain (see, *e.g.*, Bjorklund et al., 1976, 1979; Daszuta et al., 1988) or spinal cord (see, *e.g.*, Nygren et al., 1977; Nornes et al., 1983; Foster et al., 1985). The acetylcholine-esterase (AChE) staining method was similarly effective in tracing axonal projections from basal forebrain cholinergic neurons grafted to the hippocampus (see, *e.g.*, Bjorklund and Stenevi, 1977; Dunnett et al., 1982; Gage et al., 1984). The M2 and M6 antibodies, specific for mouse neurons and glia, were introduced by Ray Lund and colleagues as a tool to trace connections from mouse cells grafted to the rat brain (Lund et al., 1985; Hankin and Lund, 1987) and have since become a standard method in studies of mouse-to-rat xenografts in the brain. Other species-specific antibodies recognizing either human or pig neuronal epitopes, such as neurofilament, NCAM or Thy-1, have been effectively used for the same purpose, again in a xenograft setting (see, *e.g.*, Wictorin et al., 1990a; Stromberg et al., 1992; Isacson and Deacon, 1996).

The early studies using transmitter specific histochemical methods or immunohistochemistry with species specific antibodies revealed an impressive capacity of grafted immature neurons or neuroblasts, obtained from fetal brain, to grow axons over long distances and in a target-directed manner, extending not only within gray matter, but also along white matter tracts, such as observed from pontine noradrenergic, serotonergic or cortical neurons grafted to the lesioned spinal cord (Nornes et al., 1983; Foster et al., 1985; Li and Raisman, 1993), from striatal projection neurons grafted to the lesioned striatum (Wictorin et al., 1990b, 1991; Isacson and Deacon, 1996; Armstrong et al., 2002) and from septal cholinergic neurons grafted to the lesioned septum (Leanza et al., 1996).

The ability of grafted neuroblasts to re-establish target-specific innervation patterns in previously denervated brain regions is particularly intriguing. Some of the most interesting experiments along these lines were performed with fetal cells transplanted to the subcortically or cortically denervated hippocampus (reviewed in Bjorklund et al., 1990; Dunnett, 1991). In the adult rat hippocampus, denervated of its subcortical afferents by a lesion of the septum or the fimbria–fornix pathway, grafted fetal cholinergic, noradrenergic and serotonergic neurons were seen to reproduce innervation patterns in dentate gyrus and hippocampal subfields that mimicked closely those of the intrinsic afferents that had been removed by the fimbria–fornix lesion (Fig. 1G,H) (Bjorklund and Stenevi, 1977; Gibbs et al., 1986; Nilsson et al., 1988; Leanza et al., 1996). The graft-derived axons were shown to form functional synaptic contacts with host target neurons (Segal et al., 1985; Clarke et al., 1986), restore hippocampal acetylcholine levels and release (Nilsson et al., 1990), and ameliorate some aspects of lesion-induced deficits in hippocampus-dependent learning and memory, as observed both in rats (Dunnett et al., 1982, 1993; Gage et al., 1984; Gage and Bjorklund, 1986; Segal et al., 1987; Hodges et al., 1991) and monkeys (Ridley et al., 1992). The ingrowth of axons was blocked by the presence of an intact cholinergic innervation, and stimulated by removal of the intrinsic afferents, suggesting that axonal outgrowth from the grafted neurons was very precisely regulated by the re-innervated target (Bjorklund et al., 1979; (Lewis and Cotman, 1982). In an interesting study using microdialysis to monitor changes in release of acetylcholine in the graft-reinnervated hippocampus, Nilsson et al. (1990) and Nilsson and Bjorklund (1992) showed that the activity of the grafted septal cholinergic neurons was markedly increased during behavioral activation by sensory stimulation, or by activation of host brain afferents, suggesting that they were under regulatory control from the host brain (Fig. 1A–F).

Interestingly, the graft-derived innervation patterns differed not only between neurons of different transmitter phenotypes, but also between different subtypes of cholinergic neurons, suggesting that the growth and terminal arborization of the ingrowing axons were precisely regulated by the denervated target: each neuron type was seen to produce distinctly different innervation patterns, the ingrowth was inhibited by an intact innervation of the same type, and it was stimulated by additional denervating lesions (Gibbs et al., 1986; Nilsson et al., 1988). This is further supported by work from Geoffrey Riasman's lab, showing that the axonal connections established by transplants of fetal entorhinal cortex in the adult mouse hippocampus and dentate gyrus are restricted to the appropriate terminal zones, and that these connections are formed only after removal of the intrinsic host entorhinal afferents (Zhou et al., 1989).

Studies of graft–host connectivity using transgenic GFP-expressing reporter mice

The introduction of fluorescent reporters, such as green-fluorescent protein, GFP, has provided a new set of powerful and versatile tools to visualize and trace axonal projections derived from grafted neurons with a sensitivity and specificity that goes beyond what has been possible with classic tract-tracing techniques. The access to transgenic GFP-expressing reporter mice and stem cell lines, in particular, has made it possible to study the connectivity of intracerebral neural grafts in a new and more refined way and opened up for more detailed studies on the ability of grafted neurons to serve as tools for reconstruction of damaged brain and spinal cord circuitry. To be useful for tracing of axonal projections, however, the fluorescent reporter has to be expressed at a sufficiently high level to fill out all axonal and dendritic projections, and the expression of the reporter has to be maintained also in fully mature neurons after transplantation. Down-regulation of the reporter in the mature cells can indeed be a problem, as observed in transplants from GFP transgenic mice where the expression of GFP was driven by the chicken β-actin or the prion promoters (Eriksson et al., 2003; Kelly et al., 2007).