

● INVITED REVIEW

# Cell replacement therapy for central nervous system diseases

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## Abstract

The brain and spinal cord can not replace neurons or supporting glia that are lost through traumatic injury or disease. In pre-clinical studies, however, neural stem and progenitor cell transplants can promote functional recovery. Thus the central nervous system is repair competent but lacks endogenous stem cell resources. To make transplants clinically feasible, this field needs a source of histocompatible, ethically acceptable and non-tumorigenic cells. One strategy to generate patient-specific replacement cells is to reprogram autologous cells such as fibroblasts into pluripotent stem cells which can then be differentiated into the required cell grafts. However, the utility of pluripotent cell derived grafts is limited since they can retain founder cells with intrinsic neoplastic potential. A recent extension of this technology directly reprograms fibroblasts into the final graftable cells without an induced pluripotent stem cell intermediate, avoiding the pluripotent caveat. For both types of reprogramming the conversion efficiency is very low resulting in the need to amplify the cells in culture which can lead to chromosomal instability and neoplasia. Thus to make reprogramming biology clinically feasible, we must improve the efficiency. The ultimate source of replacement cells may reside in directly reprogramming accessible cells within the brain.

**Key Words:** *in vivo* direct reprogramming; spinal cord injury; trauma; personalized medicine; induced pluripotent stem cell; embryonic stem cells

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## Introduction

The adult central nervous system (CNS) has limited *de novo* neurogenesis (Blight, 2002; Bechmann, 2005) and only minimal capacity to replace cells lost due to tissue insult, injury or disease. This is seen in acquired and inherited brain disease (Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS)) and in CNS injury induced by trauma, vascular insult or surgery. Trauma carries the added burden of a breakdown in the blood-brain barrier which introduces systemic immunity that exacerbates parenchymal destruction. Immune suppression can slow wound progression, but this does not promote repair. Damaged axons also do not regenerate through gliotic scars or inhibitors in myelin (Filbin, 2003). Thus we rely on redundancy and rewiring of surviving circuits for partial recovery of lost function. This failure to repair likely reflects insufficient stem cells, rather than repair competence, since exogenous cells are effective in many preclinical models of genetic, chemical and traumatic brain and spinal cord injury (SCI) (Chen et al., 2007). Indeed we (Kiel et al., 2008) and others (Windrem et al., 2008) have used cell grafts to completely rescue a lethal cell autonomous neurodegenerative mouse model. Thus at present the objectives for clinical intervention in brain repair are to arrest wound progression and promote cell replacement therapy. Here we examine potential sources of replacement cells.

## Replacement Cells

For pre-clinical milestones to be translated in clinical practice,

we must first identify an appropriate graft resource. At present, the three sources include allografts of fetal brain tissue, allografts derived from pluripotent embryonic stem cells (ESCs) (Murry and Keller, 2008), and autologous neural cells generated *in vitro* by reprogramming patient-specific somatic cells such as dermal fibroblasts. Fetal brain allografts were used for PD, the first placebo-controlled neurosurgical trial in the U.S., and the cell source, fetal dopaminergic neurons, proved both ineffective and difficult to standardize (Freed et al., 2001). ESC-derived glial progenitor cells were used in a trial for acute SCI sponsored by Geron Inc. This trial was based on initially promising pre-clinical studies with CNS myelin forming oligodendroglial progenitor cell (OPC) transplants (Keirstead et al., 2005). Both pre-clinical and clinical trials with such cells have generated sometimes sensational but often controversial results (Burke et al., 2013), and the Geron trial was halted early due to cost, immune complications and apparently cystic nodule formations. Both the fetal tissue and embryonic stem (ES) derived cells have ethical limitations, both represent allografts that require immune suppression with serious side effects, and cell grafts derived from pluripotent ESCs can also carry an unacceptable risk for neoplasia. We demonstrated that therapeutic engraftment requires substantial cell numbers (Kiel et al., 2008), well within the neoplastic load limit of ESC-derived cultures (Sadowski et al., 2010). Thus grafts derived from pluripotent cells may never be considered therapeutically safe and appropriate for organ repair.

A third potential source of replacement brain cells are autol-

ogenous somatic cells genetically engineered to trans-differentiate into neural cells. The reprogramming field has now given us this novel and exciting strategy to generate ethically neutral, patient-specific replacement cells from induced pluripotent stem (iPS) cells. Yamanaka identified four factors that reprogram fibroblasts into iPS cells (Takahashi and Yamanaka, 2006), extending early work in amphibian (Gurdon and Melton, 2008) showing that cell fate can be plastic (Yamanaka, 2009). The process appears to work on any cell type (Park et al., 2008b; Soldner et al., 2009; Yu et al., 2009) including readily accessible cells such as adipose-derived mesenchymal stromal cells (MSCs), and iPS lines have now been established from many sources (Park et al., 2008a; Soldner et al., 2009). The process requires pioneer factors Oct4 and Sox2 (Takahashi et al., 2007; Smale, 2010) but can also work with Oct4 (Kim et al., 2009; Zhu et al., 2010) combined with small molecules that promote chromatin remodeling (Huangfu et al., 2008; Lin et al., 2009). Patient derived iPS cells now have potential to generate disease specific cell types that can replace animals for drug screens. However the iPS cell reprogramming is not complete (Kim et al., 2010; Lister et al., 2011) raising the concern they may not generate valid replacement cells, and since they are immune protected autologous cells their neoplastic potential will be even greater than ESC-derived allografts.

An alternative to generate autologous cells for graft therapy is to find some combination of factors that directly reprogram fibroblasts into the desired cell types and avoid the pluripotent intermediates. Trans-differentiation has been controversial and early reports were either disproved (Bertani et al., 2005; Woodbury et al., 2000), misinterpretations due to cell fusion (Lagasse et al., 2000; Wang et al., 2003) or remain unconfirmed (Jiang et al., 2002). However, Yamanaka has rejuvenated the field and recent studies now demonstrate direct reprogramming of pancreatic exocrine cells into  $\beta$ -cells (Zhou et al., 2008) and somatic fibroblasts into hepatocytes, cardiomyocytes, blood progenitors and neurons (Zhou et al., 2008; Ieda et al., 2010; Szabo et al., 2010; Vierbuchen et al., 2010; Efe et al., 2011; Pfisterer et al., 2011; Sekiya and Suzuki, 2011; Son et al., 2011; Karow et al., 2012). The process is not fully understood and to date reprogramming to generate some cell types has resulted in only partial phenotypes (Najm et al., 2013; Yang et al., 2013).

## Reprogramming Efficiency

For SCI repair three conclusions are quite clear and none are unique to SCI research. First we need a standardized source of autologous cells. Second we need rigid and objective approaches to resolve graft outcome and evaluate efficacy (Burke et al., 2013). Third, in order to generate a safe graft reagent it is imperative that we improve the efficiency of the reprogramming process. The low efficiency of reprogramming generates very few graftable cells, and the necessary mitogen amplification *in vitro* can compromise the quality control and result in karyotype abnormalities and neoplasia concerns (Miura et al., 2006; Tolar et al., 2007).

All forms of cell reprogramming suffer from low efficiencies. For iPS cell reprogramming the efficiency is commonly 0.1%. This can be improved under experimental settings by starting with fibroblasts that contain drug inducible reprogramming factor transgenes, although for these the rate remains less than 10%. Chromatin remodeling is also an early event (Simonsson and Gurdon, 2004) and a limiting factor (Luna-Zurita and Bruneau, 2013) for cell reprogramming. Small molecules that block DNA

and histone methylation, which remodel the epigenome by removing transcription repressive marks, also enhance iPS cell reprogramming (Huangfu et al., 2008; Lin et al., 2009). We recently identified a histone H1 chaperone that also promotes reprogramming (Tso et al., in preparation). This factor appears to modulate chromatin structure by relaxing condensed chromatin, as seen with other H1 modifying enzymes (Christophorou et al., 2014) and binding proteins (Philpott et al., 1991; Martic et al., 2005; Hayakawa et al., 2012). Thus chromatin remodeling factors may provide the key to optimizing the reprogramming process.

## In vivo Reprogramming

Finally, an emerging strategy to avoid long term culture of graft cells is to deliver the exogenous reprogramming factors directly into target cells *in vivo*. A graft-free reprogramming strategy would expand our paradigm for cell replacement for clinical therapies. Gene delivery methods such as viral vectors are feasible, and direct gene transduction using episomal plasmids would avoid the safety concerns and oncogenic potential of viral vectors. For *in vivo* reprogramming to work effectively it will be necessary to identify target cell populations that can serve as a resource for cell reprogramming. For neuronal cell replacement, both pericytes (Karow et al., 2012) and astrocytes (Niu et al., 2013) can be reprogrammed into induced neurons, and elevated levels of the transcription factor Sox10 can convert peripheral satellite glia into CNS-like myelinating glia (Weider et al., 2015). Another target population in the adult brain are NG2 cells (Nishiyama et al., 1996; Nishiyama et al., 2009). NG2, a transmembrane proteoglycan expressed by OPCs *in vitro*, identifies presumed myelin lineage glial progenitors *in vivo*. The adult NG2 population includes 5% of the cells in the adult brain (Nishiyama, 2007), and at least some of these may generate other cell types (Nishiyama et al., 2009). NG2 cells can generate protoplasmic astrocytes in grey matter. They can respond to extrinsic stimuli and injury. They can also respond to neurotransmitters and thus may have a role in neural transmission. While it is not clear why NG2 cells do not promote myelin replacement in injury or disease, it is clear that at least a subset of these cells are good targets for transgene delivery to regenerate myelinating oligodendrocytes *in vivo*. To achieve this we will need to identify relevant transcription factors to effectively reprogram these cells *in vivo*.

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