Novel paths towards neural cellular products for neurological disorders

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The prospect of using neural cells derived from stem cells or from reprogrammed adult somatic cells provides a unique opportunity in cell therapy and drug discovery for developing novel strategies for brain repair. Cell-based therapeutic approaches for treating CNS afflictions caused by disease or injury aim to promote structural repair of the injured or diseased neural tissue, an outcome currently not achieved by drug therapy. Preclinical research in animal models of various diseases or injuries report that grafts of neural cells enhance endogenous repair, provide neurotrophic support to neurons undergoing degeneration and replace lost neural cells. In recent years, the sources of neural cells for treating neurological disorders have been rapidly expanding and in addition to offering therapeutic potential, neural cell products hold promise for disease modeling and drug discovery use. Specific neural cell types have been derived from adult or fetal brain, from human embryonic stem cells, from induced pluripotent stem cells and directly transdifferentiated from adult somatic cells, such as skin cells. It is yet to be determined if the latter approach will evolve into a paradigm shift in the fields of stem cell research and regenerative medicine. These multiple sources of neural cells cover a wide spectrum of safety that needs to be balanced with efficacy to determine the viability of the cellular product. In this article, we will review novel sources of neural cells and discuss current obstacles to developing them into viable cellular products for treating neurological disorders.

In certain tissues or organs, such as skin, blood and gut epithelia, endogenous tissue-specific stem cells maintain homeostasis and replace cells that die due to senescence, injury or disease. In the CNS, neurogenesis constitutively occurs in the subventricular zone/olfactory bulb (SVZ/OB) and the dentate gyrus of the hippocampus [1,2], albeit on a much lower scale in comparison to the other organs. Although activated after injury in regions other than the SVZ/OB and hippocampus [3–6], CNS endogenous regeneration does not completely restore function due to the structural complexity of neural circuitry and the inadequate generation of relevant neural cells or due to the hostile microenvironment at the injury site.

Cell transplantation therapy with supportive care is safe and efficacious for the hematopoietic system. Hematopoietic stem cells can be expanded *in vitro* and once transplanted, they are able to fully restore blood tissue to an original normal state after injury [7]. Thus, cell therapy is an approach currently under investigation to restore function in many neurological disorders (reviewed in [8]).

Specific neural cell types have been derived from adult or fetal brain, from human embryonic stem cells (hESCs), from induced pluripotent stem (iPS) cells and directly transdifferentiated from adult somatic cells, such as skin cells. In diseased or injured brain, grafted neural stem cell progeny are

confronted with multiple fate decisions made by the interplay between intrinsic properties of cells and cues from the host microenvironment. Based on this interplay, grafted cells will either survive, die, proliferate, migrate or differentiate into a specific lineage. These cell fate decisions are highly orchestrated in time and space according to the local milieu. Therefore, a complete understanding of the cell source properties, cell delivery and graft–host interactions is paramount to foster functional repair of CNS tissue. Cellular products derived from hESCs are currently in Phase I clinical trials for spinal cord injury, spinal muscular atrophy and Stargardt’s macular degeneration. In addition, neural stem cells (NSCs) derived from fetal and adult CNS are in Phase I clinical trials for amyotrophic lateral sclerosis (ALS), Parkinson’s disease, ischemic stroke, Batten disease (Phase I completed), glioblastoma, spinal cord injury and Pelizaeus–Merzbacher disease (www.clinicaltrials.gov).

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This article will focus on recent advances in the generation of specific neuronal subtypes from adult somatic cells for cell therapy and drug discovery using the indirect and direct reprogramming approaches, and the challenges ahead to bring these new cellular products into the clinical trial arena.

Cellular reprogramming to the stem cell stage or iPS cell technology

In 2006, a pioneering effort by Takahashi and Yamanaka defined four genes used to reprogram a differentiated cell back to a primitive undifferentiated and pluripotent stem cell stage [9]. Similarly to pluripotent hESCs, this type of stem cell, termed iPS cells, has the capacity to differentiate into any cell type of the body. This potential opened the door to endless possibilities for cell therapy, drug screening and modeling and studying disease mechanisms [10]. This strategy of generating pluripotent stem cells from fibroblasts or other somatic cells sidesteps political, religious and ethical concerns overshadowing the use of hESCs derived from fertilized human embryos at the blastocyst stage (Figure 1). To generate iPS cells, Yamanaka and colleagues overexpressed four genes, namely *Oct4*, *Sox2*, *KLF4* and *Myc* in fibroblasts [9]. The expression of these genes regressed mature cells to an immature pluripotent stem cell stage. Given this characteristic, these cells could then be expanded over an unlimited number of passages and differentiated into specific neural cell types. Alternatively, derivatives such as self-renewable NSCs could also be isolated and perpetuated from iPS cells over many passages. Thus, a scale-up process could potentially be designed and commercial manufacturing with a determined lot size produced under current good manufacturing practices (cGMP). It has become clear that not all of the four core pluripotency genes are necessary for reprogramming. Fewer genes were required to induce precursors with a proliferative

Figure 1 (right). Sources of neuronal lineages generated by indirect and direct reprogramming of human fibroblasts.

hESC: Human embryonic stem cell; iPS: Induced pluripotent stem.

capacity [11]. NSCs, for instance, necessitated only Oct4 to regress to a pluripotent stage [12].

Early iPS cells used retroviral vectors that integrated transgene into the host genome. This approach added a significant regulatory hurdle based on the safety concern of an increased risk of mutagenesis and tumor formation [13]. The expression of this core of transcription factors is needed transiently during an initial period to drive the cell back to a precursor stage. Thus, other nonviral delivery approaches were developed, although still at low efficiency (reviewed in [14]). These approaches consist of the introduction of DNA episomal plasmid, transposon or minicircle [15–19], direct delivery of synthetic mRNAs modified to avoid the endogenous antiviral cell response [20] or treatment with reprogramming proteins [21]. Considerable work is needed to standardize the derivation techniques that will consistently lead to complete reprogramming and increase the efficiency and reproducibility.

The importance of standardizing each reprogramming approach was highlighted in a study that reanalyzed and compared published gene-expression profiles of 68 human iPS cell lines and 23 hESC lines derived in multiple laboratories [22]. This study demonstrated a strong correlation between gene-expression signatures of both the iPS cells and the hESC lines and the laboratory of origin. The implications of these differences are significant and thus rigid and standard approaches for derivation and maintenance of iPS cells will be critical for the progress of this source of neural cells.

Immunogenicity is a significant obstacle for the success of stem cell transplantation therapy. In a surprising recent study, Zhao and

colleagues reported that the expression of three genes (*Hormad1*, *Zg16* and *Cyp3a11*) confer autologous iPS cells immunogenicity and cause rejection of the transplants [23]. A successful approach would be the development of a collection of iPS cell lines carrying different MHC haplotypes that could be matched to the host recipient. This approach has recently been tested using nonhuman primate (NHP) iPS cells and demonstrated that undifferentiated NHP-iPS cells and hESCs (H9) expressed very low levels of MHC-I and no MHC-II or natural killer-receptor CD16 [24]. The NHP-iPS cells differentiated into functional dopaminergic neurons and improved the behavioral deficit in a rat animal model of Parkinson's disease 12 weeks after transplantation [24]. The authors report graft reinnervation of the striatum without tumor formation or inflammatory reactions. It will be important to try similar experiments in an autologous or syngeneic transplantation paradigm in NHP models of Parkinson's disease.

Another important application of iPS cell technology is neurological disease modeling, which has tremendous value for all stages of drug discovery and development. The generation of neurological disease-specific iPS cells has been successful, as far as the derivation, for many diseases, such as Parkinson's disease, ALS, Huntington's disease and many others (reviewed in [10,25]). However, in most of these studies the iPS cells did not display the intended disease-specific phenotype. In ALS for instance, a motor neuron disease that causes degeneration of the motor neuron and death within 5 years of diagnosis, iPS cell-derived motor neuron did not show cellular pathologies [26,27]. The onset of the disease phenotype could be due to the *in vitro* conditions or may require further cell stressors to accelerate the pathology *in vitro* [10,25]. Alternatively,

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the disease onset in motor neurons could be noncell autonomous and triggered by other cells, such as astrocytes or microglia [23–26]. Co-culture models of hESCs and diseased non-neuronal cells served the purpose in ALS modeling and drug screening [28,29]. Thus it will be a critical milestone in iPS cell disease modeling to test whether motor neuron or non-neuronal cells first develop pathologies described in ALS patients.

Cellular reprogramming to differentiated functional cell types or transdifferentiation

Recently, there have been corroborative studies conducted in many laboratories defining transcription factors that convert a somatic cell into a desired specific cell type [30]. This is a ‘short-cut’ paradigm for generating specific neural cell types, since it sidesteps the pluripotent stem cell stage. Pang and colleagues expanded on a previous study in mouse fibroblasts [31] and reported that lentiviral-mediated expression of four neuronal transcription factors, *Brn2*, *Ascl1*, *Myt1l* and *NeuroD1*, converted fetal and postnatal human fibroblasts into neurons, termed induced neuronal (iN) cells [32]. The efficiency was around 3–5% and the iN cells necessitated co-culturing with primary mouse cortical neurons for 4–5 weeks to mature and express spontaneous postsynaptic currents in 50% of the neuronal population. Similarly, Pfisterer *et al.* found the overexpression of *Brn2*, *Ascl1* and *Myt1l* was sufficient to turn human fibroblasts to functional neurons with approximately 4% efficiency in postnatal human fibroblasts at day 12 post-transduction [33]. These authors then explored the possibility of turning these human iN cells into dopaminergic neurons by the overexpression of additional transcription factors involved during development in the specification of dopaminergic neurons. Indeed, the overexpression of *Lmx1a* and *FoxA2* was sufficient to convert approximately 10% of iN cells to dopaminergic neurons. In a separate study, three transcription factors, including the neuronal-specific *Ascl1* and

dopaminergic-specific *Lmx1a* and *Nurr1* were sufficient for the conversion of adult fibroblasts derived from healthy and parkinsonian patients to dopaminergic neurons [34]. This study demonstrated that neither the pluripotent nor the neuronal stage was necessary for the induction of neurotransmitter-specific neuronal subpopulations.

The direct conversion of fibroblasts or other somatic cells to specific therapeutic neural subtypes is attractive since it may eliminate the potential for tumorigenicity. Current limitations of this technology are low efficiency and a limited proliferative ability, thus it may prove not to be sufficient for all the necessary quality assurance testing required for clinical use. In addition, and similarly to iPS cells, weeks are required to generate iN cells, which may impede acute autologous cell therapy interventions needed for stroke, traumatic brain injury or spinal cord injury. More studies aimed at improving the efficiency of conversion and cell perpetuation and investigating the impact of reprogramming on genomic and epigenomic integrity are necessary. Preclinical testing of the immunogenicity and appropriate functional integration in animal models is needed to demonstrate the validity of this source of neurons for the treatment of neurological disorders. Another disadvantage of autologous transplantation therapy for neurodegenerative diseases, such as Parkinson’s disease, is that the patient’s own cells of origin (i.e., fibroblasts) could carry the disease or be more predisposed to express anomalies, such as Lewy bodies, and succumb once grafted back to the host. Furthermore, the cost–effectiveness of patient-focused product development may prove to be uneconomical and thus may not be accessible to the general public or commercially viable.

Future perspective

Novel sources of neural cell products that involve cell reprogramming will

promote cell therapy approaches, drug development and disease modeling, as long as the reprogramming process is well defined, reproducible and leads to the disease phenotype.

The field of iPS cell research needs to address several issues to substantiate its validity as a potential source of cells for disease modeling and cell therapeutics. Improved and more efficient reprogramming approaches are in development. Likewise, the implications of gene networks and variations in identity revealed in proteomic and genomic signatures will prove therapeutically relevant. The reprogramming process to generate iPS cells is associated with genetic and epigenetic alterations (see comment in [35]). The consequences of these alterations on functional integrity of the differentiated progeny *in vivo* are unknown. Currently, hESCs have set the benchmark to guide our judgment on the validity of cell reprogramming to generate specific neural cell types for therapeutic use.

The development and advancement of direct reprogramming technology will now focus on the key criteria of stable growth rate, defined and predictable differentiation profiles and functional integration in relevant disease models in order to produce new cellular products. These novel cellular products may prove to supersede or perhaps more likely serve to complement various other sources of neural cells in translational research.

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Key points

- Specific neural cell types have been derived from adult or fetal brain, from human embryonic stem cells (hESCs), from induced pluripotent stem (iPS) cells and directly transdifferentiated from adult somatic cells, such as skin cells.
- In 2006, a pioneering effort by Takahashi and Yamanaka defined four genes used to reprogram a differentiated cell back to a primitive undifferentiated and pluripotent stem cell stage.
- Immunogenicity is a significant obstacle for the success of stem cell transplantation therapy.
- A successful approach would be the development of a collection of iPS cell lines carrying different MHC haplotypes that could be matched to the host recipient.
- Another important application of iPS cell technology is neurological disease modeling, which has tremendous value for all stages of drug discovery and development.
- The direct conversion of fibroblasts or other somatic cells to specific therapeutic neural subtypes is attractive since it may eliminate the potential for tumorigenicity.
- Improved and more efficient reprogramming approaches are in development.

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