Customized Brain Cells for Stroke Patients Using Pluripotent Stem Cells

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Regenerative medicine in stroke involves therapies that induce tissue repair and recovery. This is a distinct approach from reducing stroke damage: restoring blood flow, reducing cell death, or limiting secondary progression of injury. These 3 areas have a very concise or limited focus: restoring blood flow involves lysing or removing clots. Reducing cell death means neuroprotection. Limiting secondary damage involves modulating process of inflammation or delayed apoptosis. In contrast, tissue regeneration after stroke relates to many potential therapeutic targets, such as enhancing angiogenesis, neurogenesis, or gliogenesis; promoting axonal sprouting; stabilizing injured synaptic connections; or modulating excitatory/inhibitory balance in brain circuits. Single molecular targets may promote 1 specific tissue repair process, but clinical success is likely to occur if many of these reparative events are stimulated by 1 therapeutic treatment. This concept has informed the stem cell field in stroke. In experiments with transplantation of stem/progenitor cells in stroke, tissue repair can occur through direct formation of or replacement to neurons or glia, production of growth factors and cytokines, and stimulation of the cellular progenitors that lead to angiogenesis, neurogenesis, and gliogenesis.

Tissue repair and recovery after stroke has been shown with the first wave of studies in the field: the application of the easiest to produce stem or progenitor cells, such as adult progenitor cells (mesenchymal stromal cells, multipotent adult progenitor cells, hematopoietic stem/progenitor cells) or very early neural precursor cells that are differentiated from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs). With adult progenitor cells, isolation and expansion of the cells is relatively straightforward and application to stroke has progressed into 2 clinical trial efforts (Athersys, SanBio). The differentiation of ESCs or iPSCs into a very early neural precursor is a default cellular program and can be done with relatively simple methods. As a result, ESC- and iPSC-neuronal precursor cell (NPC) delivery into stroke models has been studied for many years. However, the field has recently advanced in its ability to take pluripotent stem cells and differentiate these into more specific neurons and glia. This review will discuss evolving approaches in stroke regenerative medicine: from ESC- or iPSC-NPC transplantation to transplants of stem cell–derived neurons or glia that are more differentiated and more closely related to the actual damaged cells in the brain after stroke. This review will cover first ESC- and iPSC-derived neuronal cells and then ESC- and iPSC-derived glial cells (Figure).

Method of Derivation of Neuronal Cells From ESCs and iPSCs

ESCs and iPSCs, being pluripotent in nature, can give rise to different types of neurons both in vitro and after intracerebral transplantation. There are several methods which have been developed to obtain neurons from these pluripotent cells. The most commonly used protocols for the generation of NPCs from pluripotent cells involve several steps, including generation of embryoid bodies and treatment with a neuroectoderm inducer retinoic acid, or by inhibition of transforming growth factor beta and BMP (bone morphogenic protein). The procedures may also involve coculturing with other cells and manipulations with gene expression.[1] These methods are often complicated and involve use of undefined culture medium with corresponding variable outcome. Neurons can be also generated by using of monolayer cultures of neural progenitors derived from pluripotent cells.[2] The resulting NPCs can be further expanded by growth factors either as attached monolayers or as floating neurospheres.

Long-term self-renewing neuroepithelial-like stem cells (lt-NESCs) can be also generated both from ESCs and iPSCs.[3] They are generated from neural rosette-like structures developed from embryoid bodies and can be continuously expanded in the presence of fibroblast growth factor 2 and epidermal growth factor. These cells have stable neuronal and glial differentiation competence with hindbrain specification. Most importantly, lt-NESCs have capacity to generate functionally mature human neurons. These cells resemble NPCs but with greater commitment in their molecular profile to neurons that, in development, will form hindbrain structures.

Different Types of Neurons Derived From ESCs and iPSCs

Pluripotent stem cell–derived neuronal progenitors can be driven with various treatments to differentiate into specific

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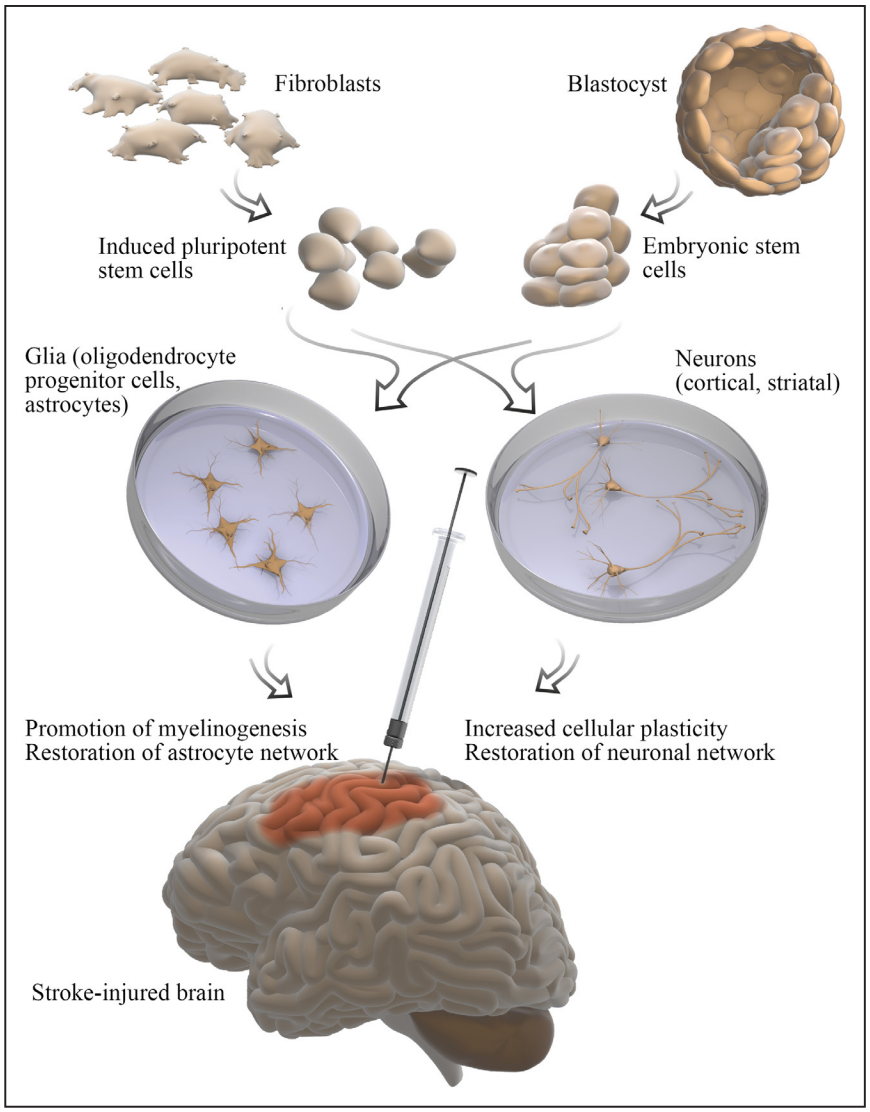

Figure. Application of pluripotent stem cells in cell therapy for stroke-injured brain. (1) Pluripotent stem cells can be derived from blastocyst (embryonic stem cells [ESCs]) or through reprogramming of postmitotic somatic cells, most commonly fibroblasts, generating induced pluripotent stem cells (iPSCs). (2) Both ESCs and iPSCs can be treated in vitro to generate glia and neurons. (3) Transplantation of glial and neuronal cells at early stages of their development into stroke-injured brain can lead to functional recovery through promotion of myelinogenesis and restoration of astrocyte network (glia) or by increasing cellular plasticity and restoring neuronal network (neurons). OPC indicates oligodendrocyte progenitor cell.

neuronal subtypes, that is, spinal motor,[4] cerebellar,[5] dopaminergic,[4] or cortical interneurons[6] and projection neurons.[7] The first convincing study demonstrating neuronal differentiation of mouse ESCs grafted in the stroke-lesioned brain was performed on rats using endothelin-induced middle cerebral artery occlusion.[8] This study showed that grafted cells can partially survive for 12 weeks after transplantation and differentiate with high yield (25%–30%) into immunohistochemically mature neurons of diverse neurotransmitter subtypes such as cholinergic (1.4%), serotonergic (1.8%), and GABAergic neurons and striatal neurons expressing substance P (1.4%) or DARPP32 (dopamine- and cAMP-regulated neuronal phosphoprotein; 6.4%). A small portion of grafted cells also differentiated into glial cells (8%). Importantly, grafted cells exhibited electrophysiological characteristics of mature neurons. Moreover, the authors also observed spontaneous excitatory postsynaptic currents in graft-derived cells indicating on their capacity to receive synaptic input. Similar results have been obtained with primate ESC-NPCs, transplanted into mice, with differentiation into several distinct subclasses of neurons and axonal extension from the transplanted cells to distant sites in the brain.[9]

Human-derived ESCs have been widely used in recent years for the generation of different types of neurons.[4,10,11] In study performed by Daadi et al,[10] ESC-derived NPCs uniformly expressed nestin, vimentin, and radial glial markers. When these ESC-NPCs were implanted into the ischemic striatum in rats, they migrated toward the lesion and enhanced recovery of motor control.[10] After transplantation, the ESC-NPCs stopped proliferating and efficiently (60%) differentiated into Tuj1$^+$ neurons, with 50% being GABAergic and only 2% glutamatergic. The same group also demonstrated[10] that the grafted cells exhibited synaptophysin staining and showed ultrastructural and electrophysiological evidence for synaptic connections. Human ESC-NPCs were also transplanted into the stroke-lesioned cortex of rats.[12] After 2 months, the majority of the transplanted cells were nestin$^+$. A portion of transplanted cells expressed neuronal markers, whereas only a few cells colocalized with astroglial or oligodendrocyte markers. In the same model of cortical stroke, human ESC-NPCs transplanted into the basal ganglia 1 week after the insult at 3 weeks after transplantation showed differentiation into neurons (65%), astrocytes (15%), and oligodendrocytes (8%).[13]

Involvement of basal ganglia lesion is very common during middle cerebral occlusion in humans. It is possible to direct human ESCs to cells with specific markers of striatal neurons that are enriched in the striatum, such as DARPP32$^+$ striatal GABAergic neurons.[11] When transplanted into the quinolinic acid–lesioned striatum of mice, these cells differentiated into DARPP32$^+$ GABAergic neurons, which projected to the substantia nigra, received glutamatergic and dopaminergic inputs, and improved impaired motor function. These data bring the opportunity to generate specific striatal projection neurons which could be used as a source for intracerebral transplantation in the stroke-damaged brain. However, predominantly striatal lesions are quite rare in stroke patients, the resulting functional impairments are mild, and spontaneous recovery is efficient.[14] Therefore, stem cell–based therapy will most likely be needed particularly for patients with cortical or combined cortico-subcortical lesions.

Although several studies used mouse iPSCs as a source for the intracerebral transplantation in mice[15] and rat[16] models of stroke, the majority of studies have been performed using human iPSCs (hiPSCs). The reprogrammed hiPSCs have been transplanted directly as iPSCs.[17] However, more often, before intracerebral transplantation in animal models of stroke, iPSCs are pre-differentiated toward NPC phenotype[18–22] or transformed into lt-NESC.[23–26] These in vitro treatments are performed with 2 main goals: to bias the fate of the cells toward a neuronal phenotype and to avoid possible tumorigenicity by removing pluripotency. Transplanted iPSCs cells can be detected by human-specific antibodies or GFP (green fluorescent protein; when iPSCs are prelabeled with this marker) up to 10 weeks after transplantation with variable survival rate between the different studies, most likely because of factors such as host strain (ie, nude rats versus immunocompetent rats) and species.[23] Although the survival time of the animals after intracerebral transplantation for either iPSC-NPC or iPSC-lt-NESCs in different studies varies from 2^{21} up to 10^{23} weeks, in all studies, grafted cells expressed early and mature neuronal markers. Among early neuronal markers, grafted cells expressed nestin,[18–20] DCX (doublecortin),[23,24,26] and βIII tubulin.[19,20] In several studies, transplanted hiPSC–derived cells differentiated into mature neurons and showed immunoreactivity for general mature neuronal markers such as NeuN,[18,22,26] MAP2 (microtubule associated protein 2),[18,20] HuD (dopamine- and cAMP-regulated neuronal phosphoprotein)[23,24,26] but also expressed more specific phenotypic makers such as gamma aminobutyric acid/glutamic acid decarboxylase 65,[18,26] glutamatergic marker kidney-type glutamate,[26] dopaminergic marker TH,[18] and maker for striatal projection neurons DARPP32.[18,26]

Different treatment of cells before or during transplantation could affect their differentiation in the host brain. The attempt by Lam et al[21] to improve survival of transplanted iPSC-NPCs to the infarct cavity of stroked mice through encapsulation in a hyaluronic acid hydrogel matrix did not lead to increased number of cells in the graft but favored DCX$^+$ neuroblast formation at 1 week after transplantation. Differentiating iPSC-lt-NESCs toward neurons with a cortical phenotype before intracerebral transplantation in stroke-subjected rats resulted in more efficient conversion to mature neurons with morphological and immunohistochemical (increased number of Tbr1$^+$ cells) characteristics of a cortical phenotype and higher axonal projection density at 2 months after transplantation.[26] These published studies clearly indicate that if hiPSCs are transformed into iPSC-NPCs or iPSC-lt-NESCs after transplantation in the stroke-damaged brain, they become prone to develop into cells with a neuronal phenotype. Differentiating these cells into more specific subtypes of neurons promotes greater integration into the brain.

In the majority of rodent studies, transplantation in stroke-damaged brain has been performed within 1 to 2 days after the insult. However, several studies have demonstrated that a positive effect of stem cell transplantation on functional recovery might occur also when cells are implanted at 1 week after stroke.[20,21,23,27] Moreover, it was shown that transplantation of human ESC–derived NPCs both in young and aged rats improved stroke-impaired behavior when delivered intracerebrally at 3 weeks after the insult.[28] Also, delayed transplantation (at 6 weeks after stroke) of NPCs derived from human fetal striatum did not influence cell proliferation, magnitude of migration, or neuronal differentiation in the grafts.[29] It is conceivable that the most suitable time for transplantation after stroke in humans will be from several weeks up to 3 months. However, this prediction needs to be supported by further experimental and clinical data.

Degree of Behavioral Improvement of ESC or iPS Neuronal Cell Transplant

The analysis of all published papers, which performed ESC-NPC and iPSC-NPC transplantation after stroke and performed the assessment of the behavioral/functional recovery, revealed that virtually in all studies some degree of improvement because of cell implantation was observed. A beneficial effect has been seen with early transplantation, <3 days from the stroke, and later transplantation times. These improvements were observed in general neurological score,[27] in motor,[12,13,19,23,24,26,27] in sensorimotor,[10,12,19,22–24,27] and in memory function tests. This general improvement with ESC or iPSC transplantation implies that there may indeed be a general effect of a progenitor cell in its action on adjacent, injured tissue. In several studies in which iPSC or iPSC-NPC transplantation did not produce behavioral recovery, these cells formed tumors.[20] It is conceivable that direct brain pathology caused by transplanted cell tumorigenesis prevented a beneficial effect on functional recovery. The formation of tumors from transplanted cells is a potential problem in all stem/progenitor therapies and a focus of the regulatory pathway of cell therapy

Mechanisms of Action of ESC- and iPSC-NPCs

The mechanisms underlying promotion of functional recovery in experimental stroke observed as a result of implantation of pluripotent stem cell–derived NPCs remain mostly unknown. Most of the studies indicate that the grafted cells promote functional improvement by mechanisms other than neuronal replacement—an effect of the transplant that is through induction of distinct tissue responses in the injured brain. More recent studies indicate that NPC transplants may differentiate into functional neurons and integrate into the poststroke brain.

In their effect in inducing changes in the injured brain, a consistent finding in the transplantation field is that the ESC- or iPSC-NPCs reduce secondary damage in stroke. After the acute cell detain in stroke, there is slowly progressive secondary

element of tissue loss in connected brain structures. Several clinical[30,31] and experimental[32] studies demonstrated secondary alterations and cell loss after stroke in the areas functionally related to the lesion site. Analysis of postmortem material from patients with middle cerebral artery infarction at least 4 months prior death demonstrates neuronal loss in the ipsilateral thalamus[31] and substantia nigra[30] and subcortical ischemic lesions induce thinning of connected cortical regions.[33] In rodent stroke models, iPSC-NPC transplantation reduced overall damage and tissue loss in the ischemic hemisphere, with transplantation both within days of the stroke or even the first week.[13,24]

A take-away point from these studies is that observed behavioral improvements seem to be related to a graft-exhibited paracrine effect in the remaining brain host tissue. The major argument in support of this assumption is that functional recovery is often observed much earlier than grafted cells differentiate to a certain phenotype and thus will be able to exhibit their respective function. Multiple mechanisms have been proposed for this paracrine effect of stem cell–mediated therapies. Among them neuroprotection, promotion of progenitor cell responses in the processes of angiogenesis and neurogenesis, and immunomodulation are most feasible mechanisms. Notably, all these mechanisms are based on the assumption that grafted cells through releasing different factors and molecules act on the surviving neurons of the host brain tissue, as well as glial and immune cells.

This paracrine effect produced by transplanted human pluripotent stem cells might be because of the secretion of plasticity-promoting trophic and other factors. Several studies implicated release of VEGF (vascular endothelial growth factor) from transplanted stem cells as mechanism for improved poststroke recovery.[16,34,35] VEGF induction in the stroke-injured brain by ESC- or iPSC-NPC transplantation may be transient, but the improved behavioral effects are long lasting.[23,24] VEGF production by the transplanted cells themselves is a second mechanism of a paracrine effect, induced by the transplanted cells. Transplantation of a fetally derived NPC produces VEGF-related effects in dendritic sprouting, axonal plasticity, and axonal transport.[36] It should be emphasized, although, that increased VEGF signaling is one possible explanation for the beneficial effects and other mechanisms or secreted factors, not explored in these studies, could be responsible for improved behavioral performance.

The plasticity of the poststroke surviving brain tissue might be also increased at cellular level through promotion of poststroke neurogenesis or effects on the immune response after stroke. iPSC-NPC transplantation in stroke promotes proliferation in the subventricular zone and migration of cells with markers of immature neurons to the site of stroke damage.[18,37] The exact cellular mechanism for enhanced poststroke neurogenesis in behavioral recovery remains unclear. An inflammation–suppression capacity has been also shown for pluripotent stem cell–derived cells in animal models of stroke, and this mechanism is widely considered as possible way for transplanted cells to promote functional recovery. Transplantation of iPSC-NPCs very early after stroke (24 hours) reduces inflammatory cytokine and chemokine production in the brain and secondary blood–brain barrier opening.[19] Early transplantation of iPSC-lt-NESC or fetally derived NPCs modulates microglial/macrophage responses to stroke[24,37] and alter the balance of pro- and anti-inflammatory cytokine signaling.

Transplanted ESC- or iPSC-NPCs may also differentiate into mature neurons and directly integrate into the poststroke brain. Neuronal integration of grafted ESC- or iPSC-NPCs injured host neural network will most likely lead to optimum functional recovery after stroke, but direct evidence that neuronal replacement really occurs is virtually lacking. However, accumulating evidence indicates on potential of grafted ESC- or iPSC-NPC-derived neurons to reconstruct neuronal circuitry. It has been shown that transplanted ESC- or iPSC-NPCs show spontaneous postsynaptic currents indicative of neurons[8,10,23] and have ultrastructural evidence of synaptic formation.[10,25] Graft-derived neurons in the cortex exhibit AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor–mediated evoked currents by stimulating a cortical region remote from the transplant[23] and respond electrophysiologically to peripheral stimulation.[25] Transplanted iPSC-NPCs generate long-distance connections, such as from striatum to globus pallidus,[23] thalamus,[9] or other distant sites.[27] In cortical stroke and iPSC-NPC transplantation, transplanted cells can extend their axons even into contralateral cortex.[26] Using rabies virus tracing of direct synaptic input, cortically transplanted iPSC-NPCs receive connections from adjacent intact cortex after stroke.[25] However, it is still unclear whether neuronal replacement and integration in injured circuitry of grafted cells contribute to the long-term recovery of impaired motor, sensory, or cognitive functions after stroke. Modern methods such as optogenetics can be used to inhibit or stimulate the activity of grafted neurons at different stages of poststroke recovery, whereas animals are performing various behavioral tasks as it has been demonstrated in animal model of Parkinson's disease.[38] This approach will be instrumental in determining the mechanisms underlying functional recovery and the significance of integration of grafted cells in host neural circuitry.[39]

Pluripotent-Derived Glial Cells

hiPSCs have been efficiently differentiated to astrocytes[40] and oligodendrocyte progenitor cell (OPCs).[41,42] Demyelinating diseases, injuries, and conditions, including pediatric leukodystrophies, white matter stroke, radiation-induced damage after cancer therapy, and spinal cord injury, are characterized by the loss or dysfunction of oligodendrocytes and the primarily death of glial cells. A more OPC- or astrocyte-based therapy is ideally suited for brain repair because of the completely different cellular constituents of all of these diseases.

Replicating the white matter ischemic damage seen in humans has proven to be relatively difficult in experimental animals. Overall, rodent stroke models have many well-recognized limits, such as differences in tolerance to cerebral edema, a small region of subcortical white matter to model lacunar infarction, and important molecular differences in thrombotic, inflammatory, and DNA repair cascades compared with humans.[43] Although it is not possible to duplicate all components of human white matter stroke in an animal model, it is essential to control infarct location. Different kinds of vasoconstrictor drugs (ie, N(5)-(1-Iminoethyl)-L-ornithine HCl or endothelin 1) are used to significantly reduce local blood flow to levels that produce ischemic injury, when injected directly

into parenchyma, and to induce precise and reproducible focal ischemic lesions in gray or white matter without disruption of the blood brain barrier. Although these white matter stroke models would not be suitable to model vasogenic edema, the histological studies show significant similarities to human white matter stroke. Axonal injury is another hallmark of white matter stroke that is replicated by vasoconstrictor-induced ischemia.[44] Compared with the rodent, the pig brain has greater anatomic and physiological similarities to humans with respect to gray to white matter composition, blood flow, gyral patterning, metabolism, and size—key factors that directly affect injury evolution, tissue recovery, and treatment development.[45] The development of primate and higher mammal stroke models is an important goal, but without institutional change in animal facilities and costs, rodent models will continue to provide the predominant basic science research into the mechanisms of neuroprotection and neural repair after stroke.

iPSC-Derived OPCs

Pre-differentiation into the oligodendroglial lineage has been shown to be more efficient for remyelination-mediated repair than grafting undifferentiated or uncommitted cells. Human pluripotent stem cell–derived OPCs are capable of rescuing brain function through remyelination in a mouse model of congenital hypomyelination,[46] promote functional recovery in a rat model of radiation-induced brain trauma,[47] and yield encouraging initial clinical results for cervical spinal cord injury.[48] Moreover, ESC-OPC transplantation is the focus of a clinical trial in spinal cord injury.[49] These results suggest that remyelination is a target for a neural repair therapy in many brain diseases and may also be a target in stroke, where white matter injury and oligodendrocyte loss are prominent.[44]

However, differentiating pluripotent stem cells along the oligodendrocyte lineage has been a long-standing challenge in the field.[46] Several protocols for the differentiation of hiPSCs to OPCs have been published.[50] The process is lengthy, usually taking >3 months. This might hinder the clinical use of a human OPC therapy, especially if the goal is to use autologous cell transplants, because the time window for beneficial cell transplantation might be shorter than the differentiation protocols. Longer differentiation times are needed for greater lineage commitment or to generate mature oligodendrocytes, which lose the ability to migrate and remyelinate spared axons.

iPSC-Derived Astrocytes

Astrocytes have a central role in brain development and function, and so have gained increasing attention as a source for a stem cell–based therapy for stroke, multiple sclerosis, congenital or early myelin loss in periventricular leukomalacia, and the hereditary and metabolic disorders of myelin loss, the pediatric leukodystrophies.[51] Astrocytes normally provide trophic and tropic support to neurons and have important functions in protecting neurons from toxic levels of glutamate and potassium. In addition, normal astrocytes have the ability to migrate along white matter tracts after transplantation into the brain; this migratory capacity may be useful in disseminating a transplant to widespread areas of the post-stroke brain. iPSC-derived astrocytes differentiated by using chemically defined, xeno-free protocols can be maintained at an immature stage in culture.[40,52] Moreover, iPSC-derived immature astrocytes can be further differentiated to astrocytes with defined mature phenotypes.[40] However, it remains unclear how precisely engrafted glial progenitors can recapitulate the pleomorphism of the host glial network they are intended to replace. In particular, the extent to which the development of an astroglial morphological and functional phenotype in the adult brain is cell-autonomous or context-dependent remains unclear. Interestingly, several studies have proven that iPSC-derived immature astroglial transplants promote myelinogenesis and improve behavioral outcome in animal models of periventricular leukomalacia. These results implicate a novel strategy for promoting myelinogenesis by iPSC-derived immature astroglia that may be extended from these nonstroke conditions into stroke.

Advantages and Limitations of ESC- and iPSC-Neural Cells

Currently, both ESC- and iPSC-derived cells are considered as potential source for cell therapy in stroke. However, there is still ethical controversy on clinical use of ESCs. Further, ESCs are by definition foreign to the transplant recipient—they are an allogeneic transplant and likely will need some degree of immunosuppression. iPSCs have an advantage when compared with ESCs by providing a potential source of patient-specific cells for transplantation. iPSCs being derived from skin biopsy have virtually no ethical concerns in contrast to ESCs obtained from human embryos. However, both viral DNA constructs, which are permanently integrated into the host genome, and the use of the c-myc oncogene as one of the transcription factors to produce iPSCs increase the chance of tumorigenicity.[53] Recently developed nonintegrating reprogramming methods based on episomal vectors, synthetic mRNAs, and Sendai viruses[54] allow efficient production of iPSCs from various somatic cells for potential future applications in clinical settings that avoid these genome integration problems. Some groups have developed iPSCs without c-myc, by using nanog and lin-28 instead[20] or only Sox2 (sex determining region Y-box 2) and Oct4 (octamer-binding transcription factor 4).[15] Such hiPSCs that are free of vectors and transgenes have been used to generate NPCs with subsequent transplantation in stroke model.[20,22] In these studies, no tumors were detected after 4 weeks[20] or 12 months[22] after transplantation in stroke-lesioned brain.

Challenges of Developing Autologous Neural Therapies

When considering the pros and cons of application of iPSCs as an autologous source for stroke patients, there are several factors which need to be taken into account (Table). First, the risk for stroke in 75 to 84 years old is 25-fold higher than the risk for 45 to 54 years old people.[55] The vast majority of stroke patients are older than 75 years, and it is unclear how reproducibly and efficiently one can generate iPSCs from aged sources. Although some studies show successful generation of iPSCs from aged humans because the major bulk of the existing preclinical studies are based on iPSCs derived from embryonic,

Table. Pros and Cons of Different Pluripotent Sources for Stem Cell Therapy in Stroke Patients

	Pros	Cons
Autologous iPSCs	No need for immunosuppression	Long time for generation, validation, and expansion
	Ethically noncontroversial	Old age of the patient as a donor for fibroblasts
Allogeneic iPSCs (HLA matched)	Minimal need (?) for immunosuppression	High expensive to generate and validate all HLA haplotypes
	Ethically noncontroversial	Need for special facilities for storage and expansion of lines
	Freely available on demand	
	Easily expandable	
ESCs	Less genetic manipulation	Ethically controversial
	Freely available on demand	
	Easily expandable	

ESC indicates embryonic stem cell; HLA, human leukocyte antigen; and iPSC, induced pluripotent stem cell.

postnatal, or young/nonaged fibroblasts, further investigations are needed. It is of great importance to determine whether iPSCs derived from aged patients are similarly beneficial for poststroke functional recovery. Second, many studies in the field have transplanted iPSC-derived cells in acute (directly after stroke) or subacute (24 hours to 1 week after onset of insult) time points, as noted above. The efficient generation and expansion of iPSCs from an aged patient's skin fibroblasts within this time frame based on existing technologies is not feasible. Currently, generation of well-characterized iPSCs, pre-differentiated toward desired a neuronal phenotype, and produced in a sufficient number of cells for transplantation might take at least 7 weeks.[20,23,26]

Recently, generation of functional neurons with different phenotype has been demonstrated through direct conversion from fibroblasts (termed induced neurons),[26,56] and this process is much faster than iPSC production. Forced expression of the 3 neurodevelopmental transcription factors Ascl1, Brn2, and Myt1l is sufficient to convert mouse fibroblasts into induced neurons with morphology and electrophysiological properties closely resembling that of mature primary neurons. Importantly, induced neurons can efficiently survive intracerebral transplantation and develop morphological properties of mature neurons.[57] However, currently, the efficiency of direct conversion is relatively low. This means that direct neuronal conversion of ESC or iPSC cells may not be a process that can be scaled up to the billions or trillions of cells that would be necessary for a clinical therapy. Small molecules can be used to improve efficiency of induced neuron cell conversion,[57] and to convert human[58] and mouse fibroblasts[59] to functional neurons, suggesting that this field may evolve as viable source for a transplantation therapy.

Isolation and validation of iPSCs and development of NPCs or lt-NESCs for individual stroke patients based on currently available methodology might be too complicated and time-consuming procedure which might fail to be useful within existing therapeutic window. However, a new compelling alternative to using patient-specific cells for transplantation could be to create an iPSC bank which can then be used for allografting trials in patients. Such a bank will provide iPSC lines generated under good manufacturing production conditions, well characterized, comprehensively tested, and cryopreserved with all potential human leukocyte antigen haplotypes matching the population of respective countries.[60] It has been reported that in nonhuman primates, autologous transplantation without immunosuppression of iPSC-derived neural cells is beneficial in terms of the immune response and cell survival compared with allogeneic grafts.[61] Importantly, the same team recently demonstrated that haplotype-matching reduces the immune response and increases the survival of grafted dopaminergic neurons in cynomolgus macaques.[62] However, MHC matching did not completely evade the immune response. Therefore, it was proposed that MHC matching might not be sufficient to avoid immunosuppression but could reduce the dose and duration of the immunosuppressive treatment. Establishment of iPSC banks has been considered in several countries including Japan, the United States, and the United Kingdom. The most advanced iPSC bank is located in Japan[63] and by 2022 is expected to have about 60 iPSC lines covering all human leukocyte antigen haplotypes for the entire population of Japan. An iPSC bank is likely to be extremely useful also for the treatment of stroke patients, considerably reducing both the costs and the time between the insult and cell transplantation.

Conclusions

Pluripotent stem cells can be differentiated into immature neurons (NPCs) and more differentiated and specific neuronal subtypes as well as astrocytes and oligodendrocyte precursor cells. Most experimental studies in stroke have been performed with transplantation of NPCs. However, transplantation of ESCs or iPSCs that have been differentiated into more committed cortical or striatal subtypes of neurons shows substantial synaptic integration into the poststroke brain and may respond to cues specific to their brain region. Transplanted NPCs and more committed or mature neurons promote repair and recovery through a paracrine effect on injured brain, reducing secondary tissue loss and promoting angiogenesis, neurogenesis, gliogenesis, and modulating neuroinflammation. Stroke damages not just neurons of course, and astrocyte and OPC therapies promote remyelination and recovery in many brain injury models, providing new directions in stroke. ESCs as a source for a cell therapy in stroke have ethical and practical limitations that may be overcome by iPSC approaches, particularly in the generation of iPSC haplobanks.

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Disclosures

None.

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