Stem Cell Therapy for Repair of the Injured Brain: Five Principles

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Abstract

Cell therapy holds great promise for regenerative treatment of disease. Despite recent breakthroughs in clinical research, applications of cell therapies to the injured brain have not yielded the desired results. We pinpoint current limitations and suggest five principles to advance stem cell therapies for brain regeneration. While we focus on cell therapy for stroke, all principles also apply for other brain diseases.

Keywords

stroke, cell therapy, regenerative therapy, iPSCs, NPCs, brain injury

Stroke is a major cause of disability and death worldwide, with no current regenerative treatments available. Stem cell–based therapies have been established for many disease areas outside the brain with remarkable success. However, the brain and the heterogenous nature of the stroke pathology pose a challenge for translating promising findings from preclinical studies into clinical reality. Here, we identify five major limitations and provide suggestions for solutions using the recent developments in the field of cellular and genetic engineering (Fig. 1).

First Principle: The Cell Source

Various cell sources are considered to accomplish successful cell therapy for brain regeneration, including adult stem cells, embryonic stem cells, or induced pluripotent stem cells (iPSCs; Zhang and others 2020). However, all cell types have shown limitations in their applicability. Clinically, the most frequently used adult stem cell source is primary mesenchymal stem cells due to their accessibility and ease of isolation. Mesenchymal stem cells may exert trophic support but do not differentiate into the neural lineage; therefore, they cannot be used as cell replacement therapy in the brain (Laso-García and others 2019). Primary neural stem cells can integrate into damaged neural networks but are rarely accessible, as they are usually extracted from the temporal lobe or subventricular zone of individuals undergoing neurosurgery for epilepsy treatment. Alternatively, neural stem cells can be derived from aborted fetal tissue and clonally expanded after immortalization (Kalladka and others 2016). Embryonic stem cells have a greater ability to differentiate into a variety of cell types and can be propagated indefinitely as compared with adult stem cells. However, they are subject to severe ethical concerns due to the requirement of an embryo, and they have an increased risk of tumor formation. The ethical concerns have been overcome with the introduction of iPSCs. iPSCs are generated by the reprogramming of somatic cells into a pluripotent embryonic stem cell–like state by the ectopic expression of various reprogramming factors (de Leeuw and Tackenberg 2019). While initial methods used the integrating lentivirus, recent developments allow for nonintegrative reprogramming via episomal vectors or the Sendai virus, which further reduce the risk of malignant cell alterations and pave the way for the clinical application of iPSCs (Hockemeyer and Jaenisch 2016). As iPSCs in cell culture can be expanded indefinitely and differentiated into principally every somatic cell type, they represent an unlimited resource of any type of human cell needed for therapeutic purposes. Before transplantation, iPSCs are usually differentiated in the desired neural cell type to promote brain regeneration. Since mature neurons with complex dendrites are

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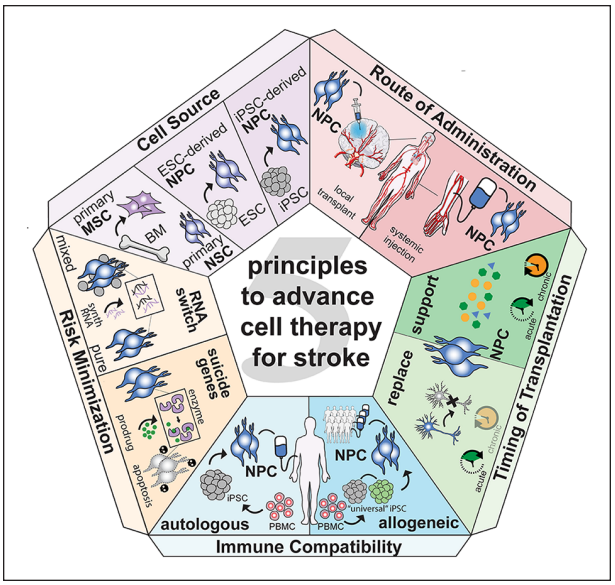

Figure 1. Five principles to advance cell therapy for stroke. BM = bone marrow; ESC = embryonic stem cell; iPSC = induced pluripotent stem cell; MSC = mesenchymal stem cell; NPC = neural precursor cell; NSC = neural stem cell; PBMC = peripheral blood mononuclear cell.

unsuitable for cell transplantations, the use of neural precursor cells is preferred. Neural precursor cells can be further customized depending on the desired precursors and the disease pathology. For instance, iPSC-derived dopaminergic progenitor cells have shown promising results in a primate model of Parkinson disease, which involves the degeneration of dopaminergic neurons in the substantia nigra (Kikuchi and others 2017). In stroke, a more heterogenous neural cell population is lost; therefore, neuronal precursor cells from a less differentiated stage promise better outcome and are used more frequently in preclinical models (Kokaia and others 2018). Transplantation of iPSC-derived cells is also associated with the risk of tumor formation (Yamanaka 2020). However, careful quality control and efficient differentiation protocols combined with novel genetic safety switch technologies (see fifth principle) can strongly reduce the tumorigenic potential of iPSC-derived cells. Thus, we propose that cell therapy based on iPSCs is the most promising approach to achieve functional brain regeneration after injury or disease.

Second Principle: The Route of Administration

Generation of the most suitable cell source for brain regeneration is of little significance if the graft cannot reach the affected brain tissue. Although current preclinical cell therapy studies favor local transplantation in the brain (Kokaia and others 2018), more than half of clinical trials prefer to administer the cells through a systemic blood injection (Negoro and others 2019). The field is faced with a dilemma: Local intraparenchymal cell transplantation promises the maximum efficacy, but the associates risks of the injection hinder its broad application. Yet, systemic injections are minimally invasive; however, most cells end up in nondesired organs, and therefore only limited therapeutic effects can be expected. Interestingly, differences in delivery can also be observed among different types of systemic injections. There is largely agreement that in rodent models of stroke, intravenous injection of cells results in 1% of cells reaching the brain (Chen and others 2001), whereas intra-arterial injection may yield 1% to 10% (Rodríguez-Frutos and others 2016). Certainly, these percentages can vary depending on the cell type, timing and severity of the brain injury, and associated blood-brain barrier (BBB) damage.

In recent years, several mechanisms have been identified that allow endogenous peripheral immune cells (e.g., neutrophils, lymphocytes, dendritic cells) to cross the intact and damaged BBB (Marchetti and Engelhardt 2020). This process involves changes in the BBB endothelium to increase the expression of adhesion molecules and reduce the presence of tight junction proteins. Simultaneously, it requires the expression of corresponding surface peptides on the entering cell to mediate rolling, adhesion, and diapedesis across the BBB (Marchetti and Engelhardt 2020). For instance, the SDF-1α/CXCR4 axis is one of the most important migratory routes across the BBB (Man and others 2012: 1). In part, these pathways have also been identified guiding grafts toward the injury site in the lesioned brain and spinal cord (Chen and others 2015; Hill and others 2004: 1). Genetic overexpression or pharmacologic induction of these peptides in the graft could therefore be a promising approach to achieve a substantial improvement in directed graft migration to the injury.

Within the brain, it is equally important to ensure retention and engraftment of the transplanted cells to the injury site. Transplanted stem cells are especially known to have a high migratory rate (up to 1.8 mm from the transplantation site within 7 d; Chen and others 2015) that may lead to undesired entrapments within the brain. For local transplantations, the most advantageous approach proved to be hydrogel encapsulation of the graft, which enhanced retention and survival with simultaneous improvement in graft maturation (Payne and others 2019). However, hydrogel encapsulation is difficult to implement for systemically applied cells due to risks of vascular occlusions. A promising alternative is to redirect the graft toward the injury-specific microenvironment by cell surface engineering. Several studies have decoded

the transcriptional and proteomic response of stroked tissue in animal models (Androvic and others 2020; Zheng and others 2020). Following the principle of chimeric antigen receptor T-cell therapy for cancer (Rafiq and others 2020), binding stroke-associated peptides may enhance the homing of grafts at the injury site and provide a more targeted and effective cell therapy after systemic application. These approaches are also currently tested in other acute injuries. For instance, coapplication of CD45-positive bone marrow–derived stem cells with a magnetic bifunctional antibody binding to CD45 and myosin light chain (present in injured cardiomyocytes) showed improved graft retention after myocardial infarction (Cheng and others 2014).

We propose that future cell delivery to the brain should be applied systemically and may rely on genetic or pharmacologic functionalization of the cells against the injury environment to improve homing and retention in the target areas while ensuring low risks during the injection. However, the optimal targets have still to be elucidated and preclinically validated. Moreover, the injury environment is highly changeable and can vary substantially within the acute and chronic phases of brain injury.

Third Principle: The Timing of Transplantation

The standard clinical procedure after stroke is to immediately restore blood flow via enzymatic medication (recombinant tissue plasminogen activator or mechanic endovascular thrombectomy; Lancet 2018). Although these procedures are highly effective, they are applicable for only a minority of stroke cases and not all patients achieve positive outcomes. It is estimated that $<$30% of patients with stroke arrive within the required narrow therapeutic time window, and of these, only 50% are eligible for acute clinical treatment (Fang and others 2010). After emergency treatment, the patient usually remains in the hospital for at least 24 h, until the condition is stable, and then starts the rehabilitation training (AVERT Trial Collaboration Group 2015). Stroked brain tissue forms a hostile environment in the acute phase, with high levels of inflammation, oxidative stress, cell death, and debris, which reduce the graft's survival chances (Shi and others 2019). The optimal therapeutic window for future cell therapy is therefore estimated to start a few days to 1 wk after stroke to ensure long-term graft survival. Therefore, most preclinical studies and some clinical designs transplant the grafts in the subacute phase after stroke (Kelly and others 2004; Kokaia and others 2018; Rust and others 2022). This phase is especially interesting for systemic cell injections, since strokes can cause a biphasic BBB opening with increased transmissibility at 4 to 6 h and 3 d after stroke; this time window increases the chances for a more efficient graft infiltration to the injured sites (Kang and Yao 2020; Weber and others 2020).

Since there are $>$100 million people worldwide living with a stroke and every second to third patient has permanent deficits (GBD 2016 Stroke Collaborators 2019), it is important to consider cell therapies for patients with chronic strokes (defined here as $>$6 mo after incident). Substantially fewer preclinical studies have investigated beneficial effects after chronic stroke in rodent models with, in part, positive results (Bhasin and others 2016; Smith and others 2012; Yasuhara and others 2009). One reason is the challenge to detect long-term deficits in stroked rodents with conventional behavioral tests; however, the recent implementation of highly sensitive 3-dimensional gait analysis is a promising refinement to quantify long-term functional changes after injury (Weber and others 2021). Yet, every second clinical trial uses patients with chronic stroke, probably also due to the clinical urgency and patient availability (Negoro and others 2019).

In the transition from acute to chronic stroke, 6 mo to 1 y after injury, usually most of the affected neural tissue and corticospinal tracts are lost; a glial scar is formed with abnormal blood supply; and many patients with impairment experience a plateau in the recovery phase (Wechsler and others 2018). While cytokine release and direct integration of the grafts have been shown to contribute to improved functional recovery after acute stroke (Llorente and others 2021; Wang and others 2016), the mechanism of action is less clear for cell therapy after chronic stroke. Enhancing functional recovery through cell replacement at this stage of tissue loss and remodeling is unlikely to be accomplished by a stem cell. However, neural and non-neural stem cell transplants have been shown to initiate angiogenesis, modify the microenvironment, enhance synaptic activity, and promote endogenous neurogenesis in the remaining brain host tissue (Kokaia and others 2018). All these observations are based on the assumption that the graft secretes regeneration- and plasticity-promoting factors that improve overall functional recovery. Among these factors, release of vascular endothelial growth factor and brain-derived neurotrophic growth factor has been identified as an important paracrine mechanism in preclinical and clinical studies (Bacigaluppi and others 2016; Bhasin and others 2016).

A takeaway point from these studies is that in the acute and subacute phases after stroke, neural stem cell therapy contributes to functional recovery through direct replacement and trophic factors. In the chronic phase, a supportive role of the cell therapy is more likely to be the predominant mechanism for improving recovery. These implications are of course also important when deciding which cell type may provide the most effective cell therapy for the individual patient.

Fourth Principle: The Immune Compatibility

Immune rejection is another critical issue in cell therapy. Autologous iPSC therapy (i.e., the application of iPSCs generated from the patient's own somatic cells) may represent the most suitable option to circumvent this problem (Yamanaka 2020). Indeed, the first study applying autologous iPSC-derived retinal cells to a patient experiencing macular degeneration did not show any signs of graft rejection (Mandai and others 2017). However, the high effort and huge costs of good manufacturing practice (GMP)–compliant production of an individual iPSC line and its quality and safety control, which are estimated at US $800,000 per cell line (Rehakova and others 2020), currently do not allow autologous therapy for a range of patients. Furthermore, the autologous approach is not applicable for acute diseases due to the time needed for clinical production of the individual's own iPSCs.

Allografts are cells or tissue from a different individual of the same species. This approach offers off-the-shelf potential, and cell banks for the storage of clinical-grade cell lines have already been established (Umekage and others 2019). A clinical trial using allogeneic iPSC-derived dopaminergic precursor cells for the treatment of Parkinson disease is currently running at Kyoto University (Takahashi 2020). However, this approach requires long-time treatment with immunosuppressants, which can have severe side effects, such as posttransplant diabetes, hyperlipidemia, neurotoxicity, or acute and chronic nephrotoxicity (Wojciechowski and Wiseman 2021). Unlike organ transplantation, the treatment with immunosuppressive drugs may be not lifelong for cell therapies. Withdrawal of immunosuppressants after 1 to 2 y have shown promising results with fetal nigral cell transplantations in patients with Parkinson disease (Hauser and others 1999).

A way to decrease allograft rejection is human leukocyte antigen (HLA) matching. HLA genes are divided into two classes: I and II. Class I consists of three major genes, HLA-A, B, and C, while class II comprises HLA-DR, DQ, and DP. HLA genes are highly polymorphic, and tens of thousands of combinations exist (Koga and others 2020). The HLA proteins are expressed on the cell surface, where they act as ligands for T cells and natural killer (NK) cells, which thereby can identify the cell as one's own body cell. The use of cells from donors homozygous for a common HLA variant can increase the immunocompatibility between graft and recipient. A study of 10,000 UK donors estimated that a panel of 10 donors homozygous for the most common HLA types provided a complete match for 37.7% of the UK population and a beneficial match for 67.4% (Taylor and others 2005). However, these numbers do not increase linearly, and many individuals, especially those with a rare HLA haplotype, will not be able to benefit from HLA matching.

A novel approach of creating iPSCs with the highest immunocompatibility is the targeted genetic modification the HLA genes, generating so-called universal cells. While a full HLA knockout would protect the graft against a T cell–mediated immune response, it would subject the graft to destruction by NK cells (Trounson and others 2019). However, HLA-knockout cells can evade NK cell surveillance by lentiviral overexpression of CD47, thereby creating hypoimmunogenic cells (Deuse and others 2019). An alternative method, avoiding transgene expression, is the retention of a single HLA-C allele in HLA-A and HLA-B knockout iPSCs (Xu and others 2019). HLA-C–retained iPSCs could evade T- and NK-cell responses in vitro and in vivo. It has been estimated that 12 HLA-C–retained iPSC lines are compatible with >90% of the world's population. Importantly, generation of immune evasive cells at the same time poses an increased risk in cases of unwanted malignant transformations or viral infections, since they may not be detected by the immune system (González and others 2020). Therefore, it is essential to develop tools for minimizing this risk, for example by genetic engineering of suicide genes.

Fifth Principle: The Risk Minimization

Cell-based regenerative therapy is not without risk. Therefore, safety measures pre- and posttransplantation are of highest importance. The scope of risks ranges from acute cerebral bleeding (at the time of stereotactic local injection), cell clotting or cell-induced embolism (for systemic injection), and functional side effects (such as seizure and involuntary movement). In the long term, the biggest concern is a malignant tumor formation from the grafted cells and the deposition of transplanted cells in undesired tissues.

To minimize these risks, iPSC-derived cells must undergo a variety of quality control experiments. This already begins with production and expansion of clinical-grade cells under GMP conditions, which requirements include, among others, working in a clean room facility and detailed logging of all production steps, as well as the use of certified and GMP-qualified xeno-free media, substances, and equipment (Gee 2018; Rust and others 2022). Quality control of clinical-grade iPSCs includes the detailed analysis of cell identity, genomic stability, pluripotency gene and marker expression, cell morphology and viability, as well as microbiological sterility (Sullivan and others 2018). However, the best quality control is useless if potentially tumorigenic iPSCs remain in the graft due to incomplete differentiation of iPSCs

into the cells of interest. Therefore, techniques have been developed to purify the differentiated cells and/or remove residual iPSCs (Fujita and others 2022; Katsukawa and others 2016). The development of GMP-compliant cell-sorting methods based on fluorescence-activated cell sorting or magnetic-activated cell sorting allows the purification of iPSC-derived cells for clinical applications. Indeed, in a current trial on patients with Parkinson disease, administered dopaminergic precursor cells were enriched through an anti-CORIN antibody with fluorescence-activated cell sorting (Kikuchi and others 2017). An alternative method represents the "RNA switch," a synthetic mRNA that contains a specific miRNA-binding domain (Fujita and others 2022). Depending on the design of the mRNA switch, binding of a miRNA can turn the switch on (mRNA is translated) or off (mRNA is not translated). By targeting cell type–specific miRNAs—such as miR-302a-5p, which is highly expressed in iPSCs—and by using an RNA on-switch encoding for barnase, a ribonuclease causing cell death, a selective removal of residual iPSCs from the culture can be achieved. However, even transplants that do not contain residual iPSCs may contain residual dividing neural stem or progenitor cells that might be of concern and have risks for graft overgrowth.

Furthermore, systemically applied cells may end up in nondesired tissues, as described in principle 2. Therefore, the establishment of safety tools posttransplantation are of highest importance in iPSC-based therapy. The development of safety switch systems enables the specific ablation of transplanted cells in case of adverse events. In the prodrug-mediated approach, grafted cells are transduced with a gene encoding for an enzyme that converts an inactive prodrug into a toxic compound (Sheikh and others 2021). The most commonly used combination is the expression of herpes simplex virus thymidine kinase and treatment with the prodrug ganciclovir, which has been shown to abolish graft-versus-host disease in patients with leukemia after infusion of suicide gene–engineered donor lymphocytes (Ciceri and others 2009). Another system is the application of a monoclonal antibody targeting a physiologic or artificial target on the grafted cell. Complement activation or antibody-dependent cytotoxicity causes the removal of the transplanted cells. Treatment with an antibody against truncated human epithelial growth factor receptor successfully eliminated chimeric antigen receptor T cells after transplantation into mice (Paszkiewicz and others 2016). A third method is to express iCasp9 (inducible caspase 9) in the cell graft. Treatment with the biologically inert substance AP1903 induces dimerization of iCasp9, leading to the graft's removal through apoptosis. All these safety switches have a distinct limitation as the respective drugs or antibodies either can or cannot cross the BBB. However, especially for the application of iPSC-derived cells for brain regeneration, it is important to have a flexibly usable system. In case of a systemic injection and the aim to remove cells that are enriched in the periphery and did not enter the brain, the prodrug/antibody should not cross the BBB. However, if the graft in the brain showed malignant transformation, a BBB-permeable prodrug/antibody would be necessary. Therefore, we propose to generate a safety switch in which the prodrug exists in two modifications: a BBB-permeable and nonpermeable version.

Conclusion

In summary, we believe that cell therapy for stroke has enormous potential to increase the therapeutic options for patients in the foreseeable future. A systemic and acute graft injection holds most promise for an effective cell therapy that is applicable for a range of patients with stroke. The genetic and pharmacologic tools are currently being developed to ensure precise targeting to the injured areas of the brain. From our perspective, the use of a "universal" iPSC-derived neural cell line that is compatible with most of the population promises greater feasibility than individualized patient-derived cell grafts. Universal cell lines would not only overcome the logistical and cost hurdles but could also be equipped with novel genetic safety switches that would substantially improve their safety profile and minimize the risk for cancerous transformations.

Declaration of Conflicting Interests

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