Cell Stem Cell

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Next steps in regenerative medicine

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https://doi.org/10.1016/j.stem.2023.04.015

Pluripotent stem cells have opened the gate to cell replacement therapy. As we inch closer to clinical application, we must improve the effectiveness of cell-based therapies. I will discuss the combination of cell transplantation with gene therapy, medication, and rehabilitation to set sight on the next frontier of regenerative medicine.

Introduction

The public has high expectations for cell transplantation in regenerative medicine, especially as scientists have made astounding progress in this area in the past few decades. Two distinct modes of action for cell transplantation have been rigorously examined: bystander effects and replacement therapy. In the former case, the transplanted cells do not survive long. They release cytokines and exosomes to mediate drug-like effects and suppress cell death, inflammation, and other detrimental effects on surviving tissues that occur following disease or injury. This is the principal mechanism of action for cell-based therapies that transplant mesenchymal stromal cells into the body. Conversely, in the latter case, the transplanted cells survive for an extended period (perhaps even for the remaining lifespan of the recipient) and restore cellular functions lost during disease or trauma. This is the aim of most cell therapies currently being pursued using differentiated cells derived from pluripotent stem cells (PSCs).

Human embryonic stem cells (ESCs) were first reported in 1998, followed by human induced pluripotent stem cells (iPSCs) in 2007.[1] PSCs have the potential to proliferate infinitely and differentiate into all the different cell types that make up the body. Furthermore, iPSCs enable autologous transplantation because they can be derived from a patient's own somatic cells. These features together have expanded the potential of regenerative medicine enormously. Indeed, clinical trials using PSCs have already been conducted worldwide for more than ten diseases, including Parkinson's disease, age-related macular degeneration, and diabetes.[1] As we attain an even greater understanding of PSCs and broaden our abilities to fashion them into various differentiated cell types for transplantation, many more clinical trials will surely follow. However, this is not the final goal but represents only the first step in establishing cell-based therapies using PSCs. For this therapeutic strategy to be accepted as a standard of care, its safety and efficacy must first be demonstrated through clinical trials. Furthermore, cell-based therapies must fit into a country's healthcare system in terms of cost, including labor costs, manufacturing scalability, and logistics. Industry, government, and academia are now partnering to address these practical questions.

Next-generation cell-based therapies

Assuming that clinical trials will continue to yield favorable results and pave the way to approval and marketing, these are merely first-generation, stem-cell-based therapies that utilize stem-cell-derived somatic cells without modification for transplantation. In this article, however, I would like to look further into the future and consider the next generation of cell-based therapies. Presently, we employ existing treatment strategies—such as medication, gene therapy, and rehabilitation with assistive medical devices—to maximize the function of preexisting cells or to prevent cell death of affected cells due to injury or disease progression. In contrast, cell replacement therapy offers an innovative approach to medical treatment: regaining lost functions by integrating the grafted cells into the host system. This expansion of treatment options will be welcomed by physicians and patients alike. To achieve this goal, I envision the next generation of regenerative medicine treatments to also combine cell transplantation with the aforementioned therapeutic strategies (i.e., medication, gene therapy, and rehabilitation with assistive medical devices) to further bolster its effectiveness and applicability (Figure 1).

Enhancement of donor cell functions

One significant way to improve cell transplantation is to enhance the functions of donor cells through gene transfer and genome editing. The key to successful cell transplantation is to increase the cellular engraftment rate, especially in allogeneic transplantation, in which one must suppress the immune response. Immunosuppressive drugs have side effects such as renal dysfunction and infection that limit their use. As such, different research groups have been trying to suppress the expression of Human Leukocyte Antigen (HLA) by genome editing to circumvent the use of immunosuppressive drugs.[2] In the case of allogeneic transplantation, immune rejection is primarily caused by the recognition of HLA class I by host immune cells. However, natural killer cells will also attack donor cells in which all HLA class I molecules have been inactivated. Therefore, methods have been devised to selectively inactivate only HLA-A and HLA-B.[3] Such universal cells can cover a large population, but the possibility of immune rejection caused by minor antigens remains.

Alternatively, a gene that inhibits cell death, such as an anti-apoptotic Bcl-2 family member,[4] could also be introduced. In cases where a genetic abnormality causes cell death, such as familial Parkinson's disease, the genetic abnormality of the donor cells must first be repaired before transplantation to avoid a

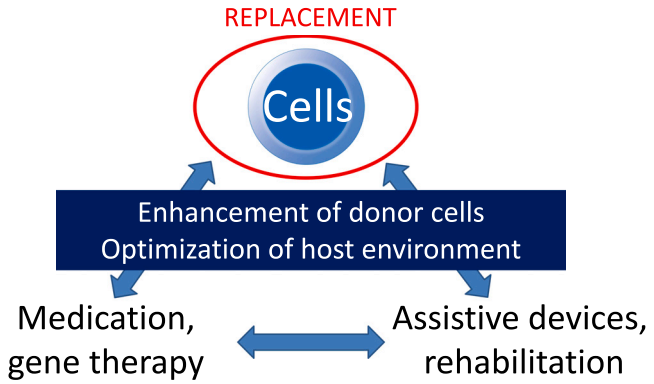

Figure 1. Concept of next-generation, cell-based therapies
Grafted cells replace lost host cells and are functionally enhanced when combined with other modalities, including medication, gene therapy, and rehabilitation.

fate similar to that of the endogenous cells. In addition to increasing cell viability, another strategy is to enhance the donor cells' function. For example, after neuronal transplantation, the grafted cells must extend axons to innervate the correct target. One possible strategy is to introduce genes that promote axonal elongation. Incidentally, although not intended for cell replacement therapy, a clinical trial is underway in which genetically modified neural progenitor cells secreting neuroprotective glial cell line-derived neurotrophic factor (GDNF) have been transplanted into the spinal cord of patients with amyotrophic lateral sclerosis (ALS).[5] Because of the blood-brain barrier, high-molecular-weight drugs cannot reach the central nervous system. Here, cell transplantation works as a drug delivery system to provide GDNF to a targeted area of the central nervous system over a sustained period.

Optimization of the host environment

The second way to improve cell transplantation is to optimize the host environment for maximal engraftment. Cell engraftment is compromised by factors such as inflammation, immune response, or poor circulation in the host organ. In addition, fibrosis and gliosis may prevent the grafted cells from functioning optimally. Therefore, the host environment is as important as the properties of the donor cells. Once again, we can utilize existing approaches, such as medication, gene therapy, and rehabilitation, to optimize the host environment. Immunosuppressive drugs are essential in allogeneic transplantation. These drugs include calcineurin inhibitors (e.g., cyclosporine, tacrolimus), antimetabolites (e.g., mycophenolate mofetil), and corticosteroids (e.g., prednisolone) that are used to promote cell survival by limiting immunoreactivity.[2] Animal experiments have, conversely, long since reported the benefits of co-administrating neurotrophic factors to promote the engraftment of neuronal transplants.[6] As described above, not only will the neurotrophic factors provide neuroprotection to the host brain, but they could also enhance the functions of the donor cells. In this context, the combination of disease-modifying therapies and cell transplantation will likely bring promising results—anti-alpha-synuclein therapy and transplantation of dopaminergic neurons to treat Parkinson's disease patients, for example.

Another viable strategy is to express guidance molecules in the host brain to promote axonal extension. To this end, we used AAV to express L1CAM—a cell adhesion molecule that functions during neurogenesis—in the mouse brain and found that it promotes axonal extension from transplanted fetal mouse cortical neurons.[7] Cortical neurons also express L1CAM, suggesting that this effect was exerted in a haptotactic manner via homophilic interactions.

Cytoprotective substances (e.g., cytokines) are secreted to enhance self-repair in damaged organs. Their identification and combinatorial therapy with cell transplantation will likely be efficacious. Depending on the substance, whether they should be administered as drugs or expressed in the host via gene therapy remains to be determined and will require case-by-case considerations. In addition, there are attempts to combine rehabilitation with cell transplantation, especially in the nervous, motor, and muscular systems. In this case, increases in local blood flow and trophic factors are expected. Furthermore, activity-dependent signaling can enhance synapse plasticity and improve neural connectivity. Inappropriate exercise, however, may promote undesirable plasticity and lead to detrimental outcomes. Therefore, patients must be guided with appropriate exercises that promote proper axon elongation and correct innervation.

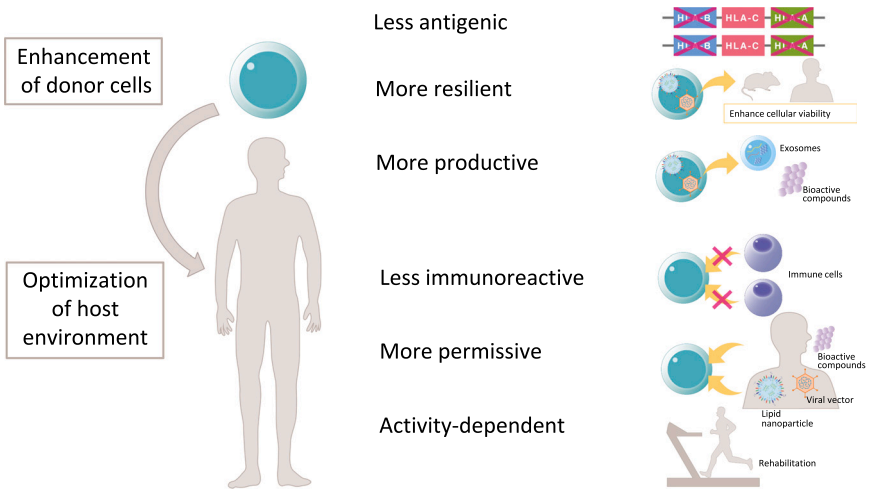

Figure 2. Strategies for successful cell-based therapies
Two aspects are key to successful engraftment: the enhancement of donor cell functions and the optimization of the host environment. Recent advances in gene editing technology will undoubtedly help fulfill both of these requirements.

Advancement of genome editing technology

As a result of recent research, genome editing technology has undergone extraordinary improvements,[8] making it possible to alter cellular functions safely and effectively. Remarkable research progress in lipid nanoparticle systems has made the introduction of RNA and small-molecule drugs—in addition to DNA—into cells feasible to program them to exhibit desired effects and functions.[9] Together with these technological advances, cell-based therapies have become more promising than ever imagined (Figure 2).

Conclusion

Regenerative medicine must go on. Lower living organisms, such as planaria and newts, can regrow whole body parts, and even higher ones demonstrate self-repair mechanisms that regulate, for instance, skin and intestinal epithelial turnover. With iPSC technology, we now possess similar powers to enable tissue self-repair and replacement through somatic cell reprogramming, directed cellular differentiation, and cell transplantation. In the future, it may become possible to repair autologous tissues without cell transplantation, but by inducing processes such as cellular rejuvenation *in situ*. As we continue to push cell-based therapies forward, why not keep an eye on current research advances—even what appears only as incremental changes at the time—with the hope that they will be the first step toward a dazzling future for regenerative medicine? We may be pleasantly surprised by how even the smallest finding can contribute to the bigger picture next time we look forward to a new horizon for regenerative medicine and reflect on its humble beginnings.

ACKNOWLEDGMENTS

I thank Dr. Kelvin Hui for his critical reading of the manuscript and Mr. Masaya Todani for his illustration.

DECLARATION OF INTERESTS

The author declares no competing interests.

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