Stem Cell Biology and Regenerative Medicine

Hossein Baharvand
Nasser Aghdami *Editors*

Regenerative Medicine and Cell Therapy

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To the memory of Dr. Saeid Kazemi Ashtiani, a wonderful colleague, a great stem cell biologist, and an inspirational advocate for stem cell research in Iran.
To our professors, teachers, students, and families.

Preface

There are numerous promising researches that show that science and medicine of the future will likely be based upon regenerative medicine and cellular therapies. Regenerative medicine is devoted to the replacement of diseased cells, tissues, or organs in congenital or acquired disease, or the repair of tissues in vivo by augmentation of natural or induction of latent regenerative processes. This new interdisciplinary field of research and clinical therapies that focus on stem cells and regenerative biology is just beginning at the dawn of the twenty-first century. In our previous book, *Advances in Stem Cell Research* (2012, Springer), we have addressed and discussed current advances and topics pertaining to stem cells, covering topics such as stem cell nano-engineering, pluripotent stem cells, and cellular reprogramming. In this book, *Regenerative Medicine and Cell Therapy*, we aim to explain clinical applications and experiences of stem cell therapy, taking into consideration neurological, ocular surface, skin, cardiac, musculoskeletal, liver and gastrointestinal diseases, and diabetes, in addition to germline and cord blood stem cells. The contributions to this book, all written by renowned experts in their respective disciplines, describe and explore various facets of regenerative medicine and cell therapy. This book will be an especially valuable resource for biomedical researchers and clinicians.

We want to sincerely thank all the authors who have contributed to this volume for their devoted efforts and their excellent contributions. We hope that you, as a reader, will enjoy this book. We are also grateful to Drs. Hamid Gourabi, Abdolhossein Shahverdi, and Ahmad Vosough Dizaj for having faith in and supporting us throughout this project. We also wish to acknowledge the great support provided by many at Humana Press. A special thank you goes to our dedicated colleagues at Royan Institute for Stem Cell Biology and Technology who, with their tireless commitment for stem cell research and therapy, have become crucial factors in encouraging us to edit this *Regenerative Medicine and Cell Therapy*. We are grateful to Zahra Maghari for her assistance with collecting the chapters and in follow-up.

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Chapter 1
Cell Therapy for Neurodegenerative Disorders

Ilyas Singec

Abstract The last decade has seen tremendous progress in stem cell biology, targeted genome editing, bioengineering, and systems neuroscience supporting the notion that cell therapy of various disorders of the central nervous system (CNS) may become clinical reality in the near future. In particular, the advent of induced pluripotent stem (iPS) cells and access to large quantities of patient- and disease-specific cellular material offers unique opportunities for developmental biology and regenerative medicine. It is now possible to investigate the molecular underpinnings of monogenic and complex human diseases using stem cell-derived neural phenotypes. Molecular insights from such studies will leverage the development of diagnostic tools, biomarkers, drugs, and cell replacement with the ultimate goal to halt or reverse the course of devastating maladies. In this book chapter, I shall discuss the opportunities and emerging challenges of stem cell-based therapies and highlight common neurological diseases that may benefit from such iatrogenic interventions.

1.1 Introduction

Owing to advances in modern medicine over the last decades, higher life expectancy has increased the proportion of the aged human population in industrialized countries. This profound demographic change allows the prognosis that chronic

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incurable disorders of the central nervous system (CNS) will have an unprecedented social and economic impact on society and the health care systems. In light of this prospect, regenerative medicine depends on innovative strategies and medical breakthroughs that can directly translate into novel therapeutics.

The human brain is characterized by enormous cellular and synaptic complexity and any attempt for repairing or replacing nervous tissue is among the most formidable goals in medicine. Neurons in the human brain are postmitotic and as old as the diseased patient [1]. In general, the human CNS has limited regenerative potential after injury and the pathobiology of neurodegenerative diseases is intricate and difficult to study. In most cases, human samples are derived from postmortem tissue with inherent problems such as poor tissue preservation and lack of standardization. In addition, postmortem specimens often reflect the end-stage of a given disease thereby limiting the study of prodromal changes.

The clinical manifestations of CNS diseases are determined by the underlying anatomical location of the lesion, the affected cell type(s), the age of onset, genetic background (familial or sporadic), and the environmental context (e.g. toxins, pesticides, cellular stressors). With regard to understanding disease etiologies, unraveling the interplay between complex genetic and environmental factors is particularly challenging. Available drugs for the treatment of neurological and psychiatric diseases are limited in that they only provide symptomatic relief but fail to target the underlying disease cause. Currently, the development of novel drugs in the pharmaceutical industry particularly for CNS diseases is experiencing major difficulties because of the poor success rate of drugs entering clinical trials [2]. This is in part due to the limited predictive value of small animal models for drug discovery emphasizing the fact that rodent models often do not recapitulate the critical aspects and peculiarities of the human condition [2–6]. Together, it is apparent that cell therapy, drug discovery, and mechanistic studies of human disease would greatly benefit from readily accessible live human neural cells amenable for basic research in a laboratory setting.

Human embryonic stem (ES) cells are the prototypical pluripotent cells and were first isolated by Thomson and colleagues [7]. Because of the ethical issues associated with the derivation of ES cells from human embryos, only a few laboratories were able to create such cell lines following strictly regulated guidelines. In addition, the dependence on limited embryo material did not allow the prospective isolation of human ES cell lines representing the large variety of familial and sporadic human diseases. The more recent discovery by Yamanaka and colleagues that human somatic cells can be reprogrammed into embryonic-like iPS cells by a few defined transcription factors represents a major breakthrough for biomedical research [8]. Nuclear reprogramming and the streamlined production of iPS cells hold great promise for clinical cell therapy and disease modeling. The availability of experimental platforms with functional human neurons derived from affected patients will greatly advance high-throughput and high-content screening efforts and drug discovery [9]. Ultimately, routine access to patient- and disease-specific iPS cells will pave the way to rigorous cell therapy and tissue engineering paradigms and realize the concept of “personalized medicine” in the twenty-first century.

1.2 Renewable Cell Sources

Pluripotent stem cells have unlimited self-renewal capacity and can generate all somatic cell types of the human body. In contrast to pluripotent cells, multipotent neural stem cells (NSCs) grown as neurospheres are characterized by more limited proliferative and developmental potentials [10, 11]. Research involving human ES cells over the last decade has helped to establish and define culture conditions that maintain human pluripotency ex vivo for extended periods of time under defined cell culture conditions [7, 12–14]. Significant progress has been made in characterizing the molecular circuitry of transcription factors, epigenetic regulators, and signal transduction pathways that maintain the pluripotent state in human ES cells [15–17]. For instance, the transcription factors OCT4, SOX2, and NANOG form an interconnected auto-regulatory circuitry controlling chromatin structure and gene expression signatures of pluripotency. In parallel, the field of nuclear reprogramming made continued progress by demonstrating that pluripotency can be induced in various somatic cells by cell fusion or improved methods of somatic cell nuclear transfer [18–21]. These approaches clearly established that factors present in the cytoplasm of pluripotent cells or mammalian oocytes can reprogram the nucleus of fully differentiated cell into an embryonic-like state. Yamanaka and colleagues were the first to demonstrate in seminal experiments that pluripotency can be induced in skin fibroblast by transient expression of four transcription factors: OCT4, SOX2, KLF4, and C-MYC [8, 22]. It was then reported that forced expression of a different set of transcription factors (OCT4, SOX2, NANOG, LIN28) can also gives rise to iPS cells [23]. Reprogramming by defined transcription factors and production of iPS cells is a robust and straightforward method and has been reproduced by a number of different laboratories exploiting viruses and virus-free gene delivery techniques. Understanding the iterative molecular processes governing successful nuclear reprogramming is currently a major effort in stem cell biology. From a developmental biology perspective it still remains extraordinary that the combined action of only four transcription factors can result in such a dramatic change of cellular fate and identity.

Detailed characterization of iPS cells has firmly established their pluripotent nature in various available assays for mouse and human cells. Tetraploid complementation is the most stringent assay for pluripotency. While this experiment is obsolete for human cells due to ethical reasons, mouse iPS cells passed this pluripotency test [24]. Ongoing work is currently revealing similarities and differences between ES and iPS cells and it is likely that these findings will reflect the biological variability among pluripotent cell lines [25]. Nevertheless, it is critical to establish the safety and genomic stability of iPS cells generated from a number of different cell types and different biological ages. The integration-free delivery of transcription factors during the reprogramming progress is critical in order to avoid insertional mutagenesis and oncogene reactivation. Rapid technical progress made over the last years indicates that virus-free reprogramming approaches will be widely used in the near future. Similarly, humanized cell culture conditions are

necessary in order to increase safety and propagate newly derived iPS cells under xeno-free conditions avoiding the risk of cross-species infections.

Allogeneic organ transplantations are traditionally associated with life-long immunosuppressive therapy. Since the derivation and application of patient-specific iPS cells holds great promise for autologous cell therapy, it is tempting to speculate that this strategy would obviate the immune rejection problem. However, a recent study reported immunogenicity of undifferentiated iPS cells upon grafting into syngeneic mice [26]. This cautionary observation is important but it remains unclear why immunogenicity was triggered in mice with the same genetic background. From a clinical perspective, undifferentiated iPS cells would not be direct candidates for cell therapy and it remains to be shown if grafting of xeno-free fully differentiated iPS cell-derived progeny can also lead to an immunological response.

1.3 Neural Differentiation Strategies for Pluripotent Cells

Scientists are now able to create iPS cells in unlimited numbers and these cells share many characteristics with human ES cells. However, our current knowledge regarding directed differentiation is still insufficient for fully utilizing pluripotent cells for clinical applications. It can be summarized that after more than a decade of research with human ES cells, robust and reproducible differentiation protocols have not been established.

Neural differentiation can be described as a process occurring in three main steps: (1) neural induction; (2) neural specification/patterning; (3) terminal differentiation. During gastrulation the three germ layers (ectoderm, endoderm, mesoderm) are formed in the early embryo. Neural induction describes the key event by which pluripotent cells enter the neural lineage [27]. Embryoid bodies (EBs) are free-floating cellular aggregates derived from pluripotent cells emulating the gastrulation process by generating precursors of the three germ layers in vitro. The use of EBs yields a heterogeneous mixture of cells in an uncontrolled fashion thereby providing lineage-restricted precursors at relatively low efficiencies. Plating of EBs on coated substrates and the subsequent spontaneous formation of neural rosettes have been used to select for neural precursors [28]. Alternatively, cell surface markers can be used to isolate neural precursors for further expansion [29]. Earlier work showed that co-culture of human ES cells with murine stromal cells can promote neural induction but this strategy is also inefficient, variable, and protracted [30]. Furthermore, high concentrations of recombinant Noggin and/or small molecule inhibitors of transforming growth factor-beta (TGFb) pathways have been shown to increase the efficiency of neural induction [31–34]. Although the more recent efforts to replace recombinant proteins by small molecules represent an important practical progress towards more defined neural induction protocols, the molecular mechanism of human neural induction as modeled by pluripotent cell lines is far from being understood. Specifically, the signal

transduction pathways and factors that positively and negatively control these processes remain elusive and have not been studied systematically.

Once formed, the neural tube undergoes specification/patterning along the rostro-caudal and dorso-ventral axis in the developing embryo. Neural patterning is guided by secreted morphogens that form gradients across the neural tissue thereby inducing specific transcription factors and neural phenotypes. The principles of neural patterning using human pluripotent cell lines are based on knowledge accumulated on animal models and require validation using human cells [27]. The potency of morphogenetic factors such sonic hedgehog (SHH), fibroblast growth factor 8 (FGF8), and retinoic acid (RA) has been confirmed in the context of human neural precursors. However, because of the large variability of published neural patterning protocols, it is important to strive for a more standardized approach for the use of morphogenetic factors (i.e. effective concentrations, treatment duration, appropriate developmental stage). Replacing recombinant proteins by small molecules is highly desirable with regard to standardization and large-scale applications and should be the main goal in all stages of neural differentiation including terminal synaptic differentiation. Robust and reproducible differentiation protocols will ensure the generation of pure populations of specific neuronal, astroglial, and oligodendroglial cells. It should be emphasized that the reproducible generation of astroglia and oligodendroglia from pluripotent cells is particularly challenging and the molecular pathways involved are poorly understood [35]. For instance, a recent study suggested that prolonged cultivation of up to 180 days is necessary to produce immature astrocytic cells from pluripotent cells [36].

1.4 Biomaterials and Disease Modeling

Stem cells in developing and adult organisms are thought to reside in highly specialized niches, which directly affect their survival, regulation, and physiological function [37]. This complex 3-dimensional microenvironment is defined by signals mediated by cell–cell contact as well as diffusible factors. There is increasing awareness that ordinary in vitro cell culture conditions fall short in providing the appropriate physico-chemical context for stem cell growth and differentiation. In fact, stem cell-based therapeutics including cell replacement, tissue engineering, and organogenesis may require the exploitation of versatile biomaterials. Hence, a more integrative approach that combines stem cell biology with other disciplines such as bioengineering will leverage effective cell-based therapies [38]. The realization of the importance of the stem cell niche has already spurred the design and application of biomaterials and experimental platforms in order to model specific aspects of the in vivo environment in high throughput [39]. The combined use of biodegradable matrices and cytokines presented to developing cells as spatially arranged gradients is likely to play important roles in tailoring personalized therapies. Similarly, to repair large parenchymal cavities after cystic

degeneration of the developing brain (e.g. stroke), it might be necessary to implant stem/progenitor cells that are seeded on biodegradable matrices [40].

There is great excitement about the potential of iPS cells not only for cell replacement but also for disease modeling. Although iPS cell-based disease modeling is a newly evolving field with many open questions, the rapid progress that has been made is remarkable and encouraging. For instance, a flurry of recent reports has demonstrated that cellular pathology of human diseases can be modeled by using disease- and patient-specific iPS cells [41–45]. The rationale behind in vitro disease modeling is to identify a cellular phenotype associated with disease and to correct this phenotype or defect by drugs, genome editing, or other interventions. Improved genetic techniques allow site-specific targeting of the human genome with zinc finger nucleases (ZFN) and transcription activator-like effector nucleases (TALENs) [46, 47]. Manipulation of defined genetic loci will facilitate the concurrent design of loss- and gain-of-function experiments using human pluripotent cells. As a consequence, disease modeling can be performed under genetically defined conditions with isogenic pluripotent cell lines further increasing the confidence into the cellular assay and the observed phenotype [48]. Nevertheless, the genetic defect alone may not be sufficient to reveal the disease phenotype during the time frame of an in vitro experiment. Pluripotent cells typically give rise to young neurons and further maturation may require prolonged cultivation, which would be a limiting factor for practical applications. To enhance synaptic differentiation, neuronal differentiation protocols may benefit from more complex cell culture conditions and biomaterials. Furthermore, since the aging process is a major risk factor for many neurodegenerative diseases, application of cellular stressors might be useful to mimic the aging process in a dish [49, 50].

1.5 Cell-based Gene and Drug Therapy

Widespread cellular engraftment into the CNS has been demonstrated for immortalized neural cell lines, progenitor cells isolated from the developing fetal brain, and neural precursors derived from human ES cells [51–55]. It is remarkable that these cells remain highly migratory upon transplantation into the normal and lesioned brain. In fact, it has been reported that grafted and endogenous NSCs preferentially home to sites of brain injury [56, 57]. Molecules secreted during the inflammatory response by immune competent cells (e.g. microglia) and astroglia are the likely chemo-attractant candidates in this process. For instance, the chemokine stromal cell-derived factor 1-alpha (SDF1-α) has been shown to play an important role in attracting NSCs to pathology [58]. Animal models of various lysosomal storage diseases and myelination defects benefit from widespread engrafted NSCs [54, 55, 59]. StemCells Inc., a California-based company, initiated Phase I clinical trials for Batten disease and Pelizaeus-Merzbacher disease using their proprietary HuCNS-SC, a cell line originally derived from human fetal brains [59].

The migratory potential of NSCs together with their amenability for genetic manipulation offers unique opportunities for combining gene and cell therapy [60].

Hence, migratory NSCs expressing foreign genes after ex vivo genetic manipulation can be exploited for targeted therapies of neurodegenerative as well as neoplastic diseases. In elegant experiments, Aboody and colleagues demonstrated the utility of migratory NSCs expressing cytosine deaminase as a vehicle for chemotherapy of invasive glioma [56]. This enzyme converts a nontoxic prodrug into a powerful chemotherapeutic compound (5-flurouracil) allowing highly selective killing of cancer cells and brain tumor-forming cells ("cancer stem cells") chased by engrafted NSCs. Glioblastoma multiforme is the most malignant human cancer characterized by highly invasive growth, lack of adequate treatment, and poor survival rate. Stem cell-based therapy holds great promise to treat high-grade human gliomas and nonneural cancers that have established brain metastasis (e.g. breast cancer). Clinical trials are currently under way investigating these therapeutic opportunities [61].

1.6 Challenges of Clinical Cell Therapy

To develop innovative therapies such as cell replacement, it is essential to perform extensive preclinical testing in animal models. Robust rodent disease models, for instance, the Parkinson's disease (PD) rat model with unilateral striatal lesions after 6-hydroxy-dopamine injection into the medial forebrain bundle, has proven highly valuable and biologically informative for cell transplantation and behavioral evaluation [62]. Once the therapeutic modality under investigation has shown salutary effects in rodents, consideration of nonhuman primate models would be the next step in the translational process towards clinical application. Careful assessment of cell therapies in nonhuman primate models is particularly important considering the enormous differences regarding organ size and anatomy between human and rodent brains.

If stem cell-based therapies are going to enter the clinical stage, preclinical tests have to provide strong evidence that benefits will outweigh the potential risks. However, some potential risks will remain until results of independent multicenter clinical trials are collected over time and conclusively analyzed with regard to safety, efficiency, and reproducibility. Ongoing efforts to coordinate and better understand fetal tissue grafting in PD will be highly valuable for future stem cell-based projects (http://www.transeuro.org.uk/). In general, it is challenging to employ a clinical cell transplantation study considering patient recruitment, sample size, and standardization of procedures. For instance, there is ongoing debate if clinical cell transplantation for PD should be carried out as open-label versus double-blind placebo-controlled trials [63].

Cell therapy of neurodegenerative disorder requires rigorous safety standards according to the guidelines of the US Food & Drug Administration (FDA). If primordial cells such as highly proliferative pluripotent stem cells are the parental source of grafted cells, any residual undifferentiated cell that remains in a cell suspension harbors the risk for tumor formation and uncontrolled growth as shown in an animal model of PD [64]. Therefore, establishing differentiation strategies

that generate homogenous cell populations at high purity and defining surface marker-based cell sorting protocols are preeminent safety requirements. The company Geron Corp., in Menlo Park (California, USA) set out to establish safety standards for human ES cell-derived oligodendrocytes for spinal cord-injured patients in a clinical trial approved by the FDA. Unexpectedly, Geron terminated recently that clinical trial because of financial considerations.

Unbiased and accurate assessment of the efficacy of cell therapy poses another challenge in the translational process. The selection of the appropriate patient cohort is as important as the timing of grafting. Depending on the disease and patient history, functional and behavioral assays should be established, analyzed, and validated over longer periods of time. In general, investigating the biology of human behavior is a challenging task. Treatment efficacy should be routinely monitored by clinical criteria, functional clinical imaging (e.g. positron emission tomography with ^{18}F-fluoro DOPA in PD) and ultimately by detailed postmortem analysis of the tissue using electrophysiological and histological techniques. Unambiguous histological detection of transplanted cells expressing a reporter gene would be ideal to evaluate the extent of cell survival, integration into the host tissue, and anatomical restoration. As mentioned above, uncontrolled growth of grafted cells can be a major problem with deleterious consequences. Another adverse event of cell therapy might be that grafted cells establish improper synaptic contacts or impair existing neural circuitries. For instance, graft-induced dyskinesia in PD has been reported as a serious complication of cell therapy [65]. It has been suggested that unwanted co-transplantation of serotoninergic cells with DA neurons and the uneven distribution of grafts forming DA releasing "hotspots" might favor the development of dyskinesia [66, 67].

Depending on the underlying disease, the most adequate route of administration and gentile cell-delivery techniques need to be determined in order to maximize survival of grafted cells and minimize injury to the host tissue [68]. Neurodegenerative diseases are often accompanied by microglia activation and chronic inflammation [69]. Although the brain is an immune privileged organ, chronic inflammation and the acute trauma caused by cell transplantation can disrupt the blood–brain barrier. Therefore, it is reasonable to believe that the host environment and the inflammatory response are important determinants of cell survival and functional integration after transplantation. Besides inflammation, the transfer of potential toxic products from host cells to grafted cells has been debated as another possible mechanism by which neurotransplantation might be compromised. For instance, there is ongoing debate about the role of alpha-synuclein inclusions and Lewy bodies that appear in grafted cells in PD patients over time [70–72]. Depending on the source of the graft, another mechanism for poor graft survival might be immune rejection and has been debated extensively in the PD literature. Immunosuppression for 6–12 months is considered to be an important parameter for successful cell therapy treatment [67]. However, the advent of iPS cell technology and generation of patient-specific cells might overcome this problem in the future. Finally, experiments in Parkinsonian rats have suggested that considering the patient's hemispheric dominance and postoperative rehabilitation are important

parameters for maximizing cell therapeutic strategies and restoring complex sensorimotor functions [62].

1.7 Neurological Diseases

Increasing evidence suggests that neuronal and synaptic dysfunction in neurodegenerative diseases are chronic and protracted processes occurring over a long period of time. Impaired neurotransmission and excitotoxic insults often precede the manifestation of clinical symptoms and can culminate in widespread apoptotic cell loss. Plausible models have been suggested that describe how chronic synaptic dysfunction can progressively develop into severe stages of neurodegeneration [73–75]. Patients suffering from PD show first clinical signs when more than 70 % of the dopamine (DA) neurons in the substantia nigra are already lost. Therefore, it is crucial to search for new diagnostic methods and detect CNS diseases in their early stages. Disease-specific markers would offer opportunities to halt or slow down disease progression at a preclinical stage. In fact, therapeutic interventions at critical early time points might even be the most advantageous strategy for correcting synaptic dysfunction by grafted cells capable of delivering growth factors and neuroprotective molecules such as brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF1), and glial-derived neurotrophic factor (GDNF).

I will next discuss prototypical neurodegenerative diseases that may benefit from cell therapies and iPS cell-based disease modeling. It is important to note that in each of these CNS diseases different cell types, anatomical compartments, and neurotransmitter systems are affected. Hence, future cell therapy strategies need to be tailored and firmly established considering the patient's history and the pathophysiology of the targeted disease. For ongoing clinical trials using NSC-based therapies see also in Aboody et al. [61].

1.7.1 Alzheimer's Disease

In 1906, the German psychiatrist and neuropathologist Alois Alzheimer described the clinical and histopathological hallmarks of a disease that was later named after him. Alzheimer's disease (AD) is the most common neurodegenerative disease worldwide. According to the Alzheimer's Association more than 5 million patients are currently diagnosed with AD in the USA alone. As a consequence, more than $170 billion are spent annually for the health care costs of AD (http://alz.org/) [76]. There is no cure for AD and effective treatment options are not available. Clinically, AD is characterized by progressive impairment of cognitive function, memory loss, and dementia. Although the precise molecular underpinnings of AD are not conclusively understood, the disease is associated with the formation

of neurofibrillary tangles ("hyperphosphorylated tau protein") and amyloid plaques and the unequivocal diagnosis can only be confirmed by directly analyzing postmortem brain tissue. Early onset and late onset types of AD can be distinguished based on the manifestation of the disease before or after the age of 60. In general, a positive family history and age are risk factors for AD. Some genetic risk factors that support the excess formation of amyloid protein in the brain ("amyloid hypothesis") have been linked to early onset AD. For instance, in Down syndrome (Trisomy 21) patients carry an extra copy of the amyloid precursor protein (APP) which leads to histopathological changes reminiscent of AD and dementia before the age of 40 [77]. Late onset or sporadic AD is the most common type and the genetic and environmental causes are not well understood. Progressive neuronal and synaptic loss in AD is widespread in neocortical and subcortical areas (i.e. basal forebrain, hippocampus, amygdala) with resultant atrophy in the frontal, temporal, and parietal lobes. Therefore, targeting multiple neurotransmitter systems and brain regions with cell therapy must be considered for AD. In addition to replacing lost neuronal cells and synaptic networks, neurotrophic factors secreted by grafted cells might contribute to the rescue of injured host neurons or modulate the rate of adult hippocampal neurogenesis [78–82]. Mild cognitive impairment occurs at early stages of AD and detecting this clinical stage might represent an opportunity for cell therapeutic interventions. On the other hand, it is plausible that the efficacy of any cell transplantation approach could be masked when treating late-stage patients with irreversible pathologies [76]. This again underscores the importance of timing and patient selection as important factors of cell therapy.

Patient-specific iPS cells have been generated from individuals with AD [83]. As an alternative to pluripotent cells, direct generation of specific neuronal subtypes by defined transcription factors has emerged as an interesting new approach [84, 85]. A recent paper has demonstrated the utility of this strategy by converting AD patient fibroblasts with specific mutations into functional neurons and demonstration of increased amyloid production by affected cells [86]. This study exemplifies the utility of direct lineage conversion for disease modeling but scalable protocols for producing a variety of cellular phenotypes are required for cell replacement. Therefore, stem cell therapy for AD is a very challenging goal due to the widespread nature of the lesions and cell loss. It is also important to consider that ongoing inflammation and toxicity due to resident amyloid plaques might impact the survival and function of cells grafted into a "hostile" environment [69].

1.7.2 Parkinson's Disease

PD is the second most common neurodegenerative disease affecting 1–2 % of the population over the age of 60. More than 95 % of the cases are sporadic and with unknown cause, whereas 1–5 % are familial and linked to genetic mutations [87]. PD leads to severe impairments of motor function reflected by the cardinal

symptoms bradykinesia, rigidity, and resting tremor. Neuropathologically, a selective and progressive loss of DA neurons of the substantia nigra pars compacta (A9) in the ventral mesencephalon results in insufficient DA concentrations in the dorsal striatum (caudate-putamen), the axonal target region of these DA neurons [88]. Figure 1.1 illustrates the complex DA system of the mesencephalon and caudate-putamen of a nonhuman primate. Although pathological changes have been reported in other systems including the olfactory bulb and enteric nervous system [89, 90], the main motor deficits in PD are owing to a deteriorated nigrostriatal projection. Treatment with L-dopa and deep brain stimulation in the subthalamic nucleus are currently the standard therapy options for PD to alleviate motor symptoms [91]. The potent drug L-dopa, which is the precursor of DA, loses its efficacy over time and may induce undesirable side effects such as involuntary dyskinetic movements [92]. Based on remarkable results with grafted fetal midbrain tissues in the early 1990s, cell transplantation and restoration of striatal DA levels has emerged as a rationale therapy [67]. A major goal of cell therapy strategies in PD is to overcome the limited availability of midbrain-type DA neurons and to circumvent the ethical issues that are inherent to working with fetal grafts. Since pluripotent cells can be propagated indefinitely under appropriate culture conditions and differentiated into DA neurons, currently this cell source appears to be the most promising approach with immediate clinical relevance.

Generation of DA neurons from mouse and human ES cells and functional grafting in rodent models of PD has been demonstrated in earlier work [30, 64, 93]. Similar transplantation studies have been recapitulated with DA neurons derived from mouse and human iPS cells [94–96]. More recently, induced DA (iDA) neurons were generated by forced expression of the transcription factors MASH1, NURR1, and LMX1A in mouse and human fibroblasts. This technology seems to allow transition from one cell type to another without reverting cells to a progenitor cell state [97, 98]. This approach is promising but the absence of self-renewing progenitor cells may pose limitations for cell replacement requiring large numbers of homogenous cell types. In addition, virus-free methods need to be established for efficient transcription factor delivery along with documentation of long-term phenotypic and genetic stability of induced neurons. Eventually, it remains to be shown if human DA neurons, derived either by reprogramming or direct lineage conversion (i.e. iDA neurons), are functional in primate models of PD and avoid adverse events such as graft-induced dyskinesia [65–67]. Optimizing cell viability and widespread synaptic integration of grafted cells are key issues that will strongly impact the outcome of transplantation studies in large animal models.

1.7.3 Huntington's Disease

Huntington's disease (HD) was first described by George Huntington in 1872 and is a devastating genetic disorder with autosomal-dominant inheritance. It typically manifests in middle age with motor and behavioral symptoms such as abnormal

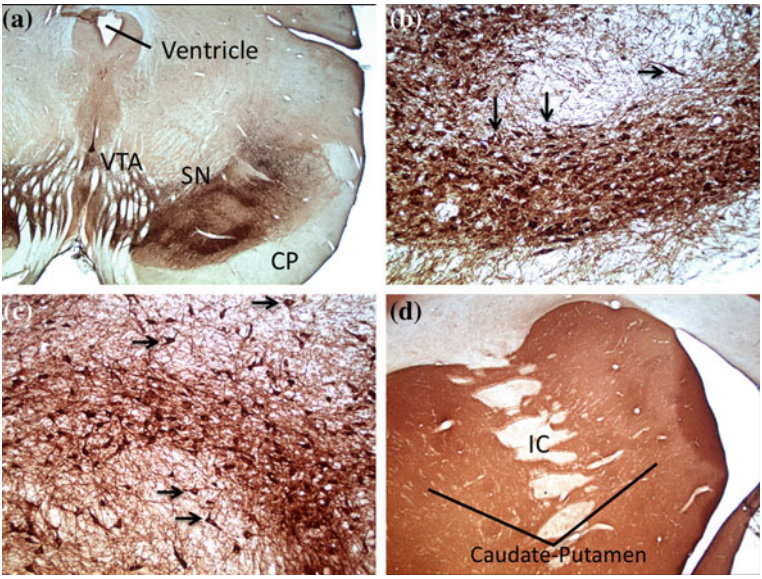

Fig. 1.1 The dopaminergic system of the midbrain (**a**–**c**) and caudate-putamen (**d**) in the monkey brain. Immunocytochemistry for tyrosine hydroxylase, the rate-limiting enzyme for dopamine synthesis, illustrates the complexity of the dopaminergic system. Dopamine neurons of the substantia nigra (arrows in **b** and **c**), which are lost in Parkinson's disease, project their axons over long distances and innervate the caudate-putamen. Histological samples are from a 4-years-old vervet monkey (*Cercopithecus aetiops*) and were kindly provided by Prof. B. Volk and Prof. D. Neumann-Haefelin, University of Freiburg, Germany. Abbreviations: *VTA*, ventral tegmental area; *SN*, substantia nigra; *CP*, cerebral peduncle; *IC*, internal capsule. Magnification: **a**, **d**, 2.5; **b**–**c**, 10

involuntary jerking and writhing movements called chorea. The progressive decline of cognitive function leads to dementia and major psychiatric problems over the time course of 10–20 years after disease onset. The genetic mutation on chromosome 4 encompasses increased CAG repeats and dysfunction of the Huntingtin (HTT) gene. The three bases cytosine, adenine, and guanine (CAG) code for the amino acid glutamine and the number of CAG repeats correlate with the severity of disease symptoms, with CAG repeats in the range of 40–120 as being pathological [99]. As a consequence, some cortical neurons and selectively vulnerable medium-sized spiny neurons expressing the neurotransmitter γ-aminobutyric acid (GABA) undergo cell death in the striatum (caudate nucleus and putamen). These striatal neurons also co-express the specific marker DARPP-32 (dopamine and cyclic AMP-regulated phosphoprotein, 32 kDa) and the neuropeptides enkaphalin or substance P [5]. Impaired BDNF provision to these striatal neurons via corticostrial projections has been shown as an additional pathogenetic mechanism [100]. The availability of iPS cells from HD patients offers novel perspectives to understand cellular and molecular disease mechanisms [83].

Currently, there is no cure for HD and treatment options for HD are primarily focused on symptomatic relief with neuropsychiatric drugs (antipsychotics, antidepressants), speech therapy, and physical rehabilitation. Rodent and nonhuman primate models of HD that are based on lesioning the striatum with excitotoxins (i.e. ibotenic acid, quinolinic acid) showed encouraging cell transplantation results and led to early phase clinical trials [101]. These open-label clinical trials were performed over the last decade and indicated slowed disease progression when grafting fetal striatal neuroblasts derived from ganglionic eminences at 7–10 weeks postconception [102]. Although beneficial effects were transient and observed in only a few HD patients, this experience is a valuable foundation for future cell replacement strategies [102–104]. At present, practical problems such as finding a renewal source for donor cells of the correct phenotype, standardization of procedures, and criteria of patient selection are the main obstacles that need to be overcome before personalized cell transplantation regimens for HD can move toward the clinic.

1.7.4 Motor Neuron Diseases

Amyotrophic lateral sclerosis (ALS), also called Lou Gehrig's disease, is a devastating neurodegenerative affliction affecting large motor neurons (MNs) localized in the primary motor cortex (upper MNs) and ventral horns of the spinal cord (lower MNs). The prognosis for ALS is poor and more than 90 % of the patients die within 2–5 years after diagnosis. The vast majority of the cases are sporadic and the underlying cause is unknown. Of the familial cases ($\sim$10 %), missense mutations in the Cu/Zn superoxide dismutase gene SOD1 have provided important insights into the pathogenesis of ALS. Oxidative stress, mitochondrial dysfunction, and excitotoxic damage are likely to be important mechanisms that ultimately lead to MN death [105, 106]. Importantly, it turned out that cell autonomous deficits of MNs are not the only pathogenetic cause of ALS. In fact, astrocytes neighboring MNs critically support their health and function but can also mediate noncell autonomous toxic effects during disease and thereby contribute to MN loss [106–109]. Functional MNs have been generated from mouse and human ES cells [110, 111]. Neuronal cells sharing molecular characteristics with *bona fide* MNs (e.g. expression of transcription factor HB9, choline acetyltransferase) were generated also by exploiting iPS cells and transcription factor-based induced neurogenesis [112, 113]. Together, these cells generated ex vivo are highly valuable for disease modeling and drug discover but it is currently unclear if MN replacement per se might be a realistic clinical approach. To restore functional motor circuits, de novo formation of long-distance axonal projections would be necessary to innervate appropriate muscle fibers in the periphery. Reconstruction of motor pathways is particularly challenging in the molecular environment provided the adult mammalian CNS. Surface molecules expressed by oligodendrocytes (e.g. Nogo proteins) inhibit axonal growth after injury [114]. Nevertheless, transplantation of MNs or glial cells that exert

neuroprotective and trophic effects might be within the realm of possibility. It is promising to see that adult mammalian motor pathways in acute rodent models of MN damage can be repaired with fetal and mouse ES cells when exploiting cell types with correct identities implanted at the appropriate time point [115–117].

Spinal muscular atrophy (SMA) is an autosomal recessive disorder representing the second most common MN disease. Four different types of SMA are known with SMA type I, also called Werdnig-Hoffmann disease, being the most severe form. Mutations of the survival of motor neuron (SMN1) gene and reduced protein expression levels are the underlying cause of SMA and severity of disease correlates with the degree of muscle weakness and early infantile onset. In contrast to ALS, MNs in the ventral horns of the spinal cord (lower MNs) are selectively affected in SMA [118]. Typically, clinical symptoms such as muscle weakness, reduced muscular tone, and progressive muscular atrophy manifest in infants. The use of iPS cells is an attractive approach to better understand disease mechanisms that lead to MN death in SMA patients. Ebert et al. [42] established iPS cell lines from patients with type I SMA, differentiated them into MNs, and observed progressive loss of these cells in vitro. This cellular assay might be useful for developing novel drugs or studying off-target effects of currently used compounds. To date, there is no effective treatment for SMA and current options are supportive in that they alleviate some disease symptoms with physical therapy and rehabilitaton, ventilators due to respiratory problems, and other types of continued medical care. Similar to the inherent problems mentioned above for ALS, cell therapy for SMA has also to consider the complex nature of motor pathways and axonal projections. It remains to be shown if replacement of MNs, supportive cells (e.g. glial cells) secreting neurotrophic factors, or gene therapeutic approaches can exert beneficial effects in SMA patients.

1.7.5 Retinal Diseases

The human retina is a highly organized multilayered tissue composed of various specific cell types (e.g. photoreceptors, interneurons, Müller glia, retinal pigment epithelium [RPE]). Because of the limited self-repair capacity of the retina, stem cell therapy is a very promising approach to restore visual function or prevent blindness [119–121]. Diseases that may benefit from stem cell therapy include retinitis pigmentosa, diabetic retinopathy, and age-dependent macular degeneration (AMD). Progress has been made in generating RPE and photoreceptors from pluripotent stem cells but these stepwise protocols need further optimization and standardization in order to increase efficiency, cell purity, and safety [122–124]. Although a cell therapy approach for highly specialized phenotypes such as photoreceptors is very challenging due to the intricate anatomical organization of the retina, these cells may indirectly benefit from the paracrine and trophic effects imparted by stem cells [121]. On the other hand, direct cell replacement and functional integration of stem cell-derived RPE is a very promising strategy. AMD is the most common cause of

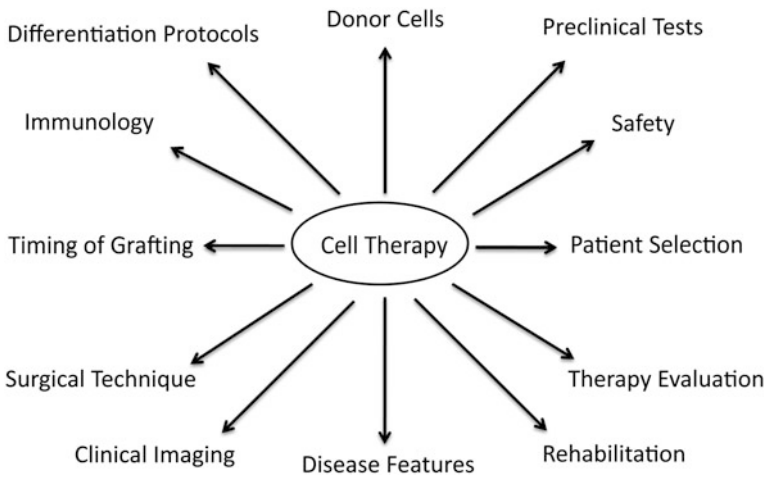

Fig. 1.2 The multiple challenges of cell therapy. Significant progress has been made over the last years regarding the various aspects of cell therapy (e.g. iPS cells as potential patient-specific donor cells). However, further multidisciplinary collaborations involving scientists and clinicians are necessary to advance cell therapies for neurodegenerative disorders

blindness in people over the age of 50. RPE cells are the specific cell type affected in this disease. Anatomically, the RPE is composed of polarized cells located between the photoreceptors and the choroid and maintain visual function by serving as a blood-retina barrier. Since RPE cells exist in a single monolayer that is surgically accessible, the efficient and controlled generation of these cells from iPS cells could pave the way to a rational autologous cell replacement therapy in the near future [121]. In 2010, the FDA has approved a clinical trial using human ES cell derived RPE cells for the treatment of dry AMD [125].

1.8 Outlook

Significant advances occurring across scientific disciplines now provide encouraging momentum for developing stem cell-based treatments for neurodegenerative disorders. Personalized therapies for intractable human diseases are no longer a distant and insurmountable scenario. It is conceivable that combined gene and cell therapy strategies might be designed and routinely offered to large patient cohorts with defined disorders that have been characterized with a number of diagnostic tools including high-throughput whole-genome sequencing, standardized cellular assays, and high-resolution functional imaging in the clinic. Integrating different cutting-edge technologies and data sharing will play crucial roles for leveraging personalized medicine. Comprehensive medical centers for cell therapy should be established in which collaboration and exchange between scientists and physicians

should speed up the efficient translation and application of breakthroughs in basic research into clinical trials. Currently, a number of challenges need to be solved as discussed and exemplified in this chapter using common neurodegenerative disorders (Fig. 1.2). For instance, to maximize the functional outcome of cell therapy it is critical to carefully select patients, generate enough numbers of relevant human cell types, and safely apply them in the appropriate time window. It is certain that the cell therapy field of the upcoming years will remain highly vibrant with regard to accumulating valuable data in clinical trials and opening up new diagnostic and therapeutic vistas.

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