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Concise Review: Stem Cells for the Treatment of Cerebellar-Related Disorders

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Key Words. Adult stem cells • Cell transplantation • Cellular therapy • Embryonic stem cells • Neural differentiation • Neural stem cell • Fetal stem cells • Stem cell transplantation

ABSTRACT

Embryonic neural transplants have become clinically relevant over the past 25 years for their possible application in the treatment of cerebellum-related neurodegenerative diseases. While highlighting the important role that fetal neural progenitors have in meeting these challenges, we define rationales for all types of cell therapy involving adult stem cells as well as human embryonic stem cells (hESC) and human induced pluripotent stem (iPS) cells. The recent advances in the field of hESC and iPS cells, including their capacity for differentiation toward regional specific neural lineages, could open a new era of transplantation in cell-based therapy for cerebellar ataxias. STEM CELLS *2011;29:564–569*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

To date, there is no treatment that can cure or substantially prolong the life span of individuals affected by cerebellar disorders, such as hereditary ataxias, that include the autosomal dominant spinocerebellar ataxias (SCAs), autosomal recesive, or X-linked form of disease [1, 2]. Stem cell-based reparative approaches have been applied to many disorders during the last two decades, including cerebellum-related disorders. These cells are defined as precursor cells that have the capacity to self-renew and generate multiple mature cell types.

Rewiring the Cellular Loss in Cerebellar Disorders

The unique and relatively simple architecture makes the cerebellum a good candidate for studying the intrinsic and environmental parameters influencing regenerative processes triggered by the injected cells. The cerebellum is involved in the coordination of voluntary motor movement, balance and equilibrium, and muscle tone. Thus, this central nervous system (CNS) structure is trilaminar and organized in a point-to-point manner representing a favorable ground for investigating whether neural replacement could be an effective strategy for re-establishing neural circuits. The brain is composed of a limited number of neural subtypes: Purkinje cells (PC), Golgi cells, granule cells (GC), stelatte cells, and basket cells, and acts as a coordination center responsible for the fine-tuning of body movement and balance [3].

The PCs are among the most distinctive neurons in the brain and also of the cerebellar cortex. The remaining four types of nerve cells constitute local interneurons. PCs, as the only projection neurons, use GABA as their neurotransmitter and therefore exert inhibitory effects transmitting signals from the cerebellar cortex to the deep cerebellar nuclei [4]. The afferent innervations of PCs originate from the inferior olivary nucleus on the contralateral side of the brainstem through climbing fibers [5] (Fig. 1). Other afferent impulses of PCs are received through the mossy fibers that arise from the pontine nuclei, spinal cord, vestibular nuclei, etc., forming excitatory synapses with the GCs and the cells of the deep cerebellar nuclei and noradrenergic outputs of axons originating in the dorsal part of the nucleus, locus coeruleus [6] (Fig. 1).

Cerebellar GCs are very abundant in the human brain, [7] exerting excitatory effects on their targets. Their dendrites receive excitatory input from mossy fibers and inhibitory input from Golgi cells, whereas unmyelinated axons rise vertically splitting off the vertical branch into two horizontal branches giving these neurons a specific "T" shape. These horizontal branches form parallel fibers that further form synapses with the dendritic trees of PCs [7].

Author contributions: S.E.: conception and design, data analysis and interpretation, manuscript writing, final approval of manuscript, other (perform experiments and graphic design); V.M.-M.: other (perform experiments); M.G.-H.: other (perform experiments and graphic design); M.S. and S.S.B.: conception and design, manuscript writing, final approval of manuscript.

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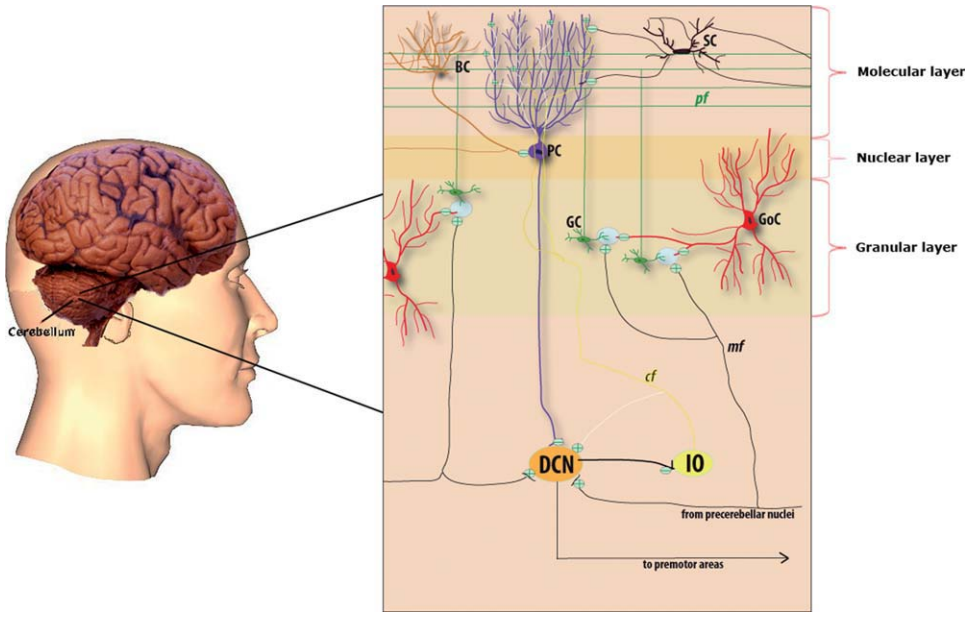

Figure 1. Cellular organization of cerebellar cortex. The Purkinje cells (PC) form dendritic trees receive the incoming excitatory afferents signals from inferior climbing fibers and parallel fibers (pf). Basket cells and stellate cells provide inhibitory input to the PC. PC sends inhibitory projections to the deep cerebellar nuclei (DCN). Granule cells (GC) send axons to molecular layer splitting in two branches horizontally to form a pf. These cells receive inputs from mossy fibers that come from precerebellar nuclei. Golgi cells make inhibitory synapses with GC dendrites. Abbreviations: BC, basket cells; cf, climbing fibers; DCN, deep cerebellar nuclei; GC, granule cells; GoC, golgi cells; mf, mossy fibers; PC, Purkinje cells; pf, parallel fibers; SC, stellate cells.

Cerebellar ataxia may originate from a large variety of heterogeneous hereditary and nonhereditary diseases. Besides the clinical signs produced by various disturbances in extracerebellar areas, a combination of cerebellar motor symptoms like instability of posture and gait, incoordination, atactic reaching and grasping, tremor, dysmetria, muscular hypotonia, and various impairments in fine motor movements [8], are common to all these disorders. All disturbances in the cerebellar cortex are derived either from the altered function or number of PCs or both. As the normal synaptic action of PCs on their target neurons is inhibitory [9], cerebellar pathology will lead to alterations in the inhibitory action of the cerebellar cortex on the deep cerebellar and vestibular nuclei increasing the firing of these neurons. The loss of inhibitory input impairs normal activity of deep cerebellar neurons that gives rise to cerebello-thalamic, cerebello-rubral, cerebello-vestibular, cerebello-reticular, and vestibulo-spinal projections thus impairing motor performance. The re-establishment of this PC inhibitory function is the major challenge of stem cell therapy.

Autosomal dominant cerebellar ataxias are an important group of hereditary diseases that includes a variety of SCAs, [1–7] and a group of episodic ataxias (reviewed in [10, 11]). The massive death of PCs is also characteristic of other hereditary and sporadic cerebellar atrophies, including Holmes type of familial cerebello-olivary atrophy, late type of cerebellar atrophy (Marie-Foix-Alajouanine), recessively inherited Fridreich's ataxia, and Ataxia-telangiectasia (A-T) [12]. The nonhereditary ataxia can include any type of focal lesion of the cerebellum, such as ischemia, brain tumor or multiple sclerosis, developmental malformations, cerebellar injuries, causing degeneration not only of PCs but also of GCs and other cells that form cerebellum. The PCs are also particularly vulnerable to alcohol and their massive loss is the hallmark of alcoholic cerebellar atrophy [13].

One of the most studied hereditary ataxia is Ataxia-telangiectasia, the disorder that causes progressive neurodegeneration due to the loss of PC in the cerebellum. This disorder is inherited as autosomal recessive and involves ataxia-telangiectasia mutated gene, responsible for progressive neurological impairment [14].

Restoring already lost neuronal functions in cerebellum could be accomplished by direct replacement of cerebellar circuits in rat model of ataxia with the new cerebellar neuronal progenitors derived from stem cells. These transplanted cells have to differentiate into mature neurons, especially PCs, and also have to be recognized and connected by host CNS and extend axons to form point-to-point neuronal junctions.

Fetal Stem Cells

One of the possible therapeutic tools in the treatment of cerebellar ataxias is a cell transplantation therapy using fetal stem cells isolated directly from fetal neuroectoderm. The main advantage of fetal cells, mostly obtained as primordial cerebellar tissue grafts, is that these cells are usually committed at their site of origin. Because of their multipotency, these cells normally complete their differentiation in the host, guided with extrinsic information provided by the recipient environment. The major disadvantage of these cells is the limited propagation, a requisite for cell-based therapy. The fetal neural grafting in various ataxia murine models of the disease has been extensively studied over the last few decades (for reviews [15, 16]) with the main focus on fate restriction and developmental potential of cerebellar progenitors as well as the role of specific regional brain environment in the determination of the fate of cerebellar progenitors. The most important studies were performed by different groups mostly using mouse cerebellar mutant model involving PC degeneration (*pcd*), first described by Mullen et al. [17], and characterized by massive postnatal degradation of PCs or weaver mutant (*wv:wv*) [2, 12, 15, 16, 18–29]. To re-establish the functional circuits in damaged *pcd* cerebellum, intracerebellar grafts derived from the wild type mouse embryo were transplanted into the mutant animals. The results of all these studies can be summarized in the following findings:

(a) The transplanted embryonic cells engraft and migrate reaching molecular layer in the host cerebellum [18, 19, 24]. (b) The afferent inputs from host's parallel and climbing fibers to grafted PC were detected by electrophysiology [20]. In summary, fetal grafts achieved high level of engraftment and migration to the adult cerebellar cortex especially in molecular layer achieving typical PC phenotype but failed to reach and establish inhibitory input to host deep cerebellar nuclei [24].

To re-establish the lost behavioral function in cerebellar ataxia animal models, functional connections between the host deep cerebellar nuclei and the grafted cells have to be achieved. The results were improved by grafting fetal cerebellar progenitors directly to deep nuclei [22, 27, 29] resulting in robust PC axonal innervations of deep nuclei with the formation of synaptic contacts and the partial enhancement of behavioral response [28]. Successive studies have indicated that implantation of fetal progenitors can reverse a large number of symptoms (both motor and cognitive) caused by PC loss of various kinds [12, 15, 21, 22, 25, 27–29].

The plethora of studies performed over decades on mouse fetal tissue led to the accumulation of important data regarding the cerebellar developmental events, differentiation, and implantation of grafted fetal cells for creating successful clinical application of neural transplantation.

There is only one study where human fetal neural tissue was used in treatment of a patient with human cerebellar disorder (A-T) [30]. Although the outcome was not encouraging as the patient developed a tumor, this does not mean that stem cell transplantation therapies have to be abandoned but that further work on safety of these therapies has to be undertaken.

Adult NSCs

Adult neural stem cells (NSCs) are usually derived from the subventricular zone lining the lateral ventricles of the brain and the subgranular zone in the dentate gyrus of hippocampus [31]. Apart from their capacity to grow in vitro their most important features are self-renewal and multipotentiality.

Transplantation of NSC produced only a small number of desired neurons and resulted in poor behavioral recovery when used in noncerebellar neurodegenerative diseases such as in Parkinson disease animal model [32]. By contrast, transplantation of these cells to animal models of the transgenic SCA1 gave encouraging results [33]. Grafted SCA1 animals have shown improved motor skills when compared with sham-treated controls [33]. This study, together with studies on other animal models such as multiple sclerosis [34], indicates that transplanted adult NSC could be beneficial for some neurodegenerative disorders. This kind of approach is obviously not feasible for clinical practice because of poor availability of human material for cell transplantation. An alternative strategy of adult NSC exploitation is in their endogenous stimulation and in situ differentiation into cerebellar cells.

Another pending issue regarding adult NSC is their identification in humans. The presence of NSCs in the adult rodent cerebellum has already been discovered [35]; however, the presence of NSCs in human adult cerebellum has not yet been confirmed.

Embryonic Stem Cells

A major disadvantage of most adult and fetal stem cells is their limited expandability and differentiation capacity. Although these cells are limited to differentiate into different cell types from their tissue of derivation, embryonic stem cells (ESC) are pluripotent cells that can be propagated in vitro for a long period and represent a theoretically inexhaustible source of precursor cells that could be differentiated into any cell type including neuronal and glial fate cells. ESCs represent a promising source from which we can study neural development or treat neurological disorders [36–39]. The ability to proliferate with no limitation and differentiation to the cells that originate from all three germ layers makes them an ideal source for cell replacement therapy.

Human ESCs (hESCs) derived specific neural progenitors are applied in clinical trials. The first cell line of oligodendrocyte progenitors derived from hESC (GNROPC1) has been approved by the U.S. Food and Drug Administration for phase I clinical trials in the treatment of spinal cord injury (SCI) (http://clinicaltrials.gov). The success of the ESC therapy in the treatment of cerebellar disorders depends on efficient differentiation of ESC into functional progenitor cells with specific cell fates and their delivery to specific cerebellar regions. Neural progenitors derived from ESCs have to display morphological and functional characteristics of their embryonic counterparts, and the proper timing of the generation of different types of neurons and glia cells. Recent studies showed that mimicking in vivo signaling pathways can generate functional cells with cerebellar characteristics from murine ESC, which express specific cellular expression markers and engraft after cell transplantation [40, 41]. Little information is available regarding the mechanisms of neural patterning and specification in the human developing cerebellum. We have recently shown that application of inductive signals involved in the early patterning in the cerebellar region of the neural tube followed by bone morphogenetic protein factors, mitogens, and neurotrophins directs hESC differentiation into functional cerebellar cells (Supporting Information Fig. 1) [36]. The majority of generated progenitors showed a GC T-shaped polarity phenotype, expressing markers specific for GCs (Supporting Information Fig. 1) [36]. We also observed the expression of the most important markers for PCs and interneuron and glial markers. The yield of PCs and GCs was higher in comparison to another protocol using murine ESC [41]. This is the first study showing the functional activity of cerebellar cells derived from hESC assessed by electrophysiological recording. Our results confirmed that neural differentiation mechanisms of murine ESC can be efficiently applied on hESC faithfully mimicking molecular and cellular mechanisms involved in cerebellar development. As most of the cerebellar ataxias are related to PC loss, further investigation has to be oriented toward designing new protocols for efficient differentiation of hESC to higher yield of PCs. Also, cell transplantation studies are necessary as a prerequisite for future applications of these cells in preclinical models of cerebellum-related diseases.

iPS Cells As a Further Step?

A serious disadvantage of ESC is their allogenicity which requires immunosupression during cell therapy. To overcome this disadvantage, scientists were searching for an alternative strategy to create patient-specific pluripotent cells with properties similar to ESC. Induced pluripotent stem (iPS) cells provided new hope for creating a patient-friendly pluripotent cell that can differentiate to any cell of the human body. After the successful creation of iPS cells from murine cells, Takahashi et al. [42] revolutionized stem cell research by generating human iPS cells by forced expression of four transcription factors, namely Oct3/4, Sox2, Klf4, and c-Myc, in human fibroblasts. These cells exhibit the properties of hESC, such as morphology, proliferation, gene expression, and teratoma formation in immuno-deficient mice [42].

Although iPS cells-derived neural subtypes have not yet been applied on any experimental model of cerebellar ataxia, a number of research groups have been evaluating neural differentiation potential and function of human iPS cells in neurodegenerative disease animal models [43, 44]. In our hands, results indicate that iPS cells can be differentiated toward cerebellar-like cells using the same differentiation strategy as the

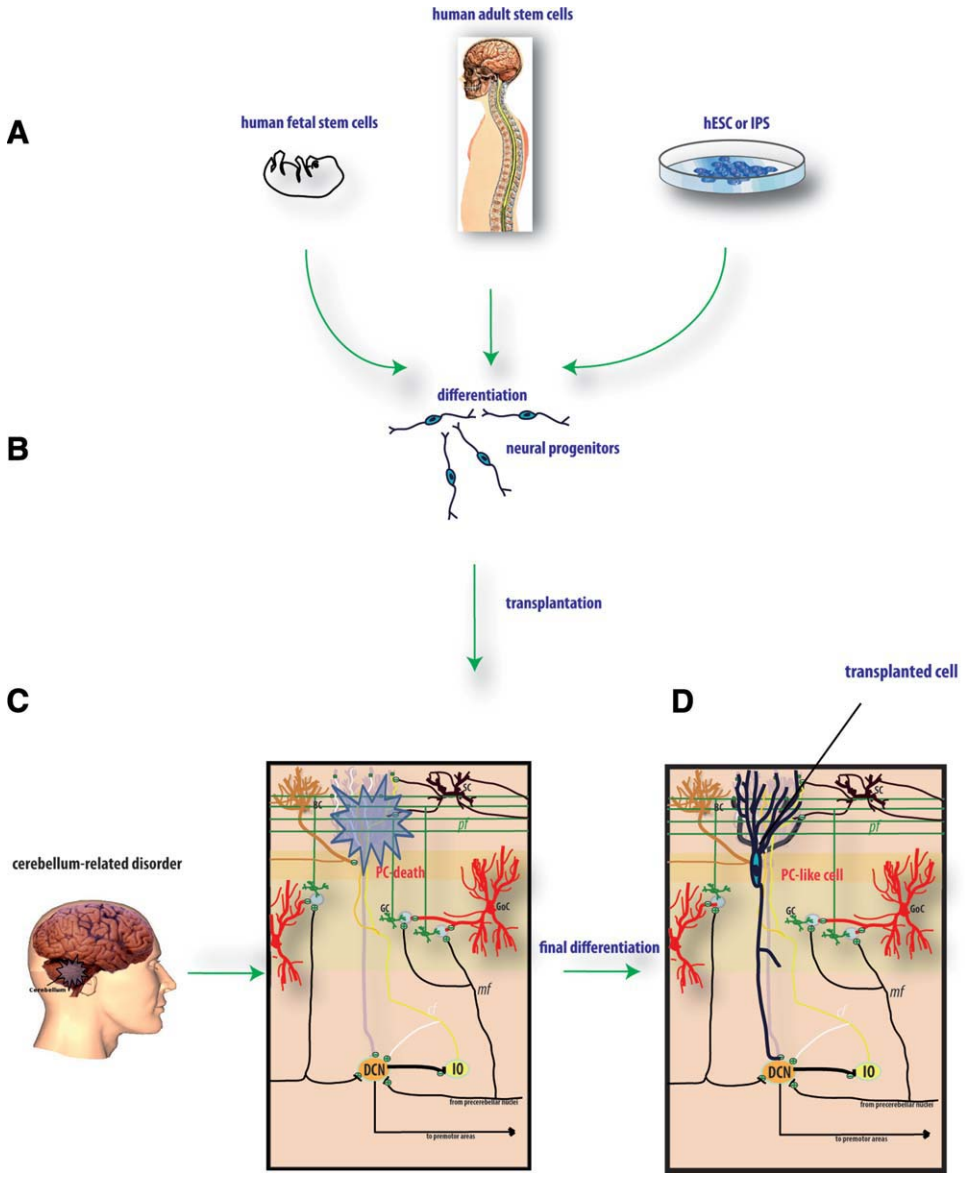

Figure 2. Possible stem cell therapies for the future treatment of cerebellar disorders. **(A):** Different sources of stem cells, that is, fetal embryonic stem cell (ESC), adult stem cells, human ESCs, and induced pluripotent stem cells, can be differentiated toward neural progenitors **(B)**. Transplantation of these progenitors in human cerebellar-related disease where Purkinje cell (PC) loss occurred **(C)** can replace damaged PCs. The progenitors migrate and re-establish PC function in the defected cerebellar area **(D)**. Abbreviations: BC, basket cells; cf, climbing fibers; DCN, deep cerebellar nuclei; GC, granule cells; GoC, golgi cells; hESC, human embryonic stem cell; IO, inferior olivary; iPS cells, induced pluripotent stem cells; mf, mossy fibers; PC, Purkinje cells; pf, parallel fibers; SC, stellate cells.

one applied to hESC (Erceg et al., unpublished results). Even though iPS cells can be directed to neural precursors and further differentiate toward regional specific CNS cell types in vitro, the clinical application of iPS cells is still far in the future. An important drawback for their clinical application is the use of retroviral vectors that include oncogenes in current reprogramming methods, which may themselves cause cancer by integrating into the genome. New studies are offering an alternative by using nonintegrating genetic episomal vectors [45] or small molecules and cytokines [46]. So far, small molecules and cytokines have not been successful in reprogramming human somatic cells. Instead, nonintegrating methods such as direct delivery of reprogramming proteins [47] or synthetic RNA [48] have been used for successful generation of human iPS cells. Recently, Tsuji et al. [44] have shown that different iPS cell clones have different in vitro neural differentiation potential and teratoma formation even after efficient recovery of behavioral function indicating that safety screening of different iPS cell clones has to be performed before their clinical application. In addition, feeder-free and xeno-free culture conditions have to be established for differentiation of these cells toward neural cells [49]. In summary, to introduce iPS cells for human regenerative therapy for cerebellar disorders, a number of important issues have to be addressed: (a) choosing appropriate adult cells for more efficient reprogramming; (b) use of xeno-free and virus-free conditions for generating patient specific iPS cells; (c) designing animal-free and feeder-free protocols for more efficient differentiation of iPS cells toward high yield of desired regional specific neurons and glia; (d) improving rigorous safety requirements to exclude tumorigenicity after transplantation to the patients.

Direct Neural Differentiation

One key disadvantage of iPS cells (and hESC) is the formation of tumors in the host. Therefore, the differentiation protocols for generation of neurons of interest need to be adapted to eliminate any trace of iPS cells or hESC. The pluripotent stem cells tumorigenicity can be overcome using the strategy of Vierbuchen et al. [50], in which the authors combined three well known transcription factors, *Ascl1*, *Brn2*, and *Myt1l*, for direct conversion of mouse embryonic and postnatal fibroblasts into functional neurons called induced neuronal (iN) cells, without passing through pluripotent stem cell stage. Derivation of iN cells from non-neural lineages could provide a new tool for the treatment neurodegenerative diseases; however, the concern remains regarding the reprogramming factors which remain in the cells.

Other Sources of Pluripotent Stem Cells

An alternative stem cell source for autologous transplantation is being found from many tissues, but one of the most important is a source of ESC-like cells called human spermatogonial stem cells [51] generated from adult human testes. Besides other differentiation potentials of these cells, a recent study showed that these cells can be efficiently converted to neurons and glia [52] by opening the possibility for their use in future treatment of neurodegenerative diseases.

The bone marrow stem cells particularly mesenchymal stem cells (MSC) and umbilical blood cells (UBC) represent another alternative source of cells for patient specific cell replacement therapy in degenerative disorders, including neurodegenerative diseases. In the recent study, Jones et al. [53] injected mouse MSC in the cerebellum of newborn Lurcher mutant mice, an animal model for cerebellar ataxia characterized by postnatal death of PC. Indeed, the transplanted cells grafted, migrated, and improved locomotor function probably providing trophic and neuroprotective effects to existing PCs [53]. Human UBC can be an effective cellular source for cell transplantation in some neurodegenerative disorders especially in SCI [54] and may play an important role in the future treatment of many neurodegenerative disease including ataxias.

Conclusion and Future Perspectives

With the aim of establishing a functional cerebellum in a patient affected by loss of PCs by stem cell derived neural grafting, it is obligatory to reconnect afferent and efferent PC inputs with "point-to-point systems." Which type of stem cells is most appropriate for future human cell therapy of cerebellar disorders is difficult to say at the moment (Fig. 2). Replacement therapy based on intracerebellar implantation of differentiated stem cells is still far from clinical trials. Nonetheless, the last three decades were extremely rich in experimental research of cerebellar grafting using fetal stem cells. Intracerebellar implantation of fetal tissue appears, at this time, to be the experimental therapeutic strategy closest to clinical trials, because these cells have been widely investigated in animal models of cerebellar ataxia. However, the difficulty in obtaining sufficient quantities of human material for transplantation represents a serious obstacle for the use of adult stem cells in regenerative therapy.

The animal models of cerebellar ataxia faithfully mimic human pathophysiology. It is considered that the *wv* mutant may be important disease model for GC loss as it is the case in certain types of hereditary degenerative cerebellar atrophy such as the GC hypoplasia in humans [10, 55] or olivopontocerebellar atrophy. The *pcd* mutant represents an animal model related more to PC loss such as cerebello-olivary atrophy [12, 28]. Although the results obtained using cell therapy in animal models can provide correlations with human cerebellar disorders, future clinical trials will give us a more clear evidence of how successful the cell therapy is in cerebellar disorders.

hESC research has identified these cells as a promising source for cell therapy promoting their application in clinical trials in humans for several neurodegenerative diseases. Our contribution in designing efficient protocols for differentiation of hESC toward cerebellar neurons and glia opened the possibility for testing these cells in animal models of cerebellar ataxia. This could herald the start of a new era for investigation of the regenerative potential of these cells, despite their disadvantages due to ethical restrictions and possible rejection by patients lacking immune suppression.

Considering the simplicity and high efficiency of generating patient specific pluripotent stem cells, iPS cells represent the most promising candidate for future regenerative therapy. However, a comprehensive evaluation of these cells is still in its early days. iPS cells offer the option of autologous transplantation, eliminating the problem of host rejection and offering at the same time high propagation efficiency, a requisite for cell-based therapies. The discovery of iPS cells does not mean that hESC research has to be abandoned. Because of many similarities and differences between hESC and iPS cells, it is very important to simultaneously compare their potential to differentiate toward cerebellar progenitors, regenerate the affected cerebellar tissue, and improve the locomotor function. The accelerated research in the field of stem cell technology gives hope that efficient cell-based therapy in cerebellar ataxia diseases will become a reality in the near future.

Acknowledgments

This work was supported by funds for research from "Miguel Servet" contract of Instituto de Salud Carlos III of Spanish Ministry of Science and Innovation (to S.E.), Fund for Health of Spain PI10-01683 (to V.M.M.), and Junta de Andalucia PI-0113-2010 (to S.E.).

Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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