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Concise Review: Modeling Multiple Sclerosis With Stem Cell Biological Platforms: Toward Functional Validation of Cellular and Molecular Phenotypes in Inflammation-Induced Neurodegeneration

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ABSTRACT

In recent years, tremendous progress has been made in identifying novel mechanisms and new medications that regulate immune cell function in multiple sclerosis (MS). However, a significant unmet need is the identification of the mechanisms underlying neurodegeneration, because patients continue to manifest brain atrophy and disability despite current therapies. Neural and mesenchymal stem cells have received considerable attention as therapeutic candidates to ameliorate the disease in preclinical and phase I clinical trials. More recently, progress in somatic cell reprogramming and induced pluripotent stem cell technology has allowed the generation of human "diseased" neurons in a patient-specific setting and has provided a unique biological tool that can be used to understand the cellular and molecular mechanisms of neurodegeneration. In the present review, we discuss the application and challenges of these technologies, including the generation of neurons, oligodendrocytes, and oligodendrocyte progenitor cells (OPCs) from patients and novel stem cell and OPC cellular arrays, in the discovery of new mechanistic insights and the future development of MS reparative therapies. STEM CELLS TRANSLATIONAL MEDICINE *2015;4:252–260*

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory neurodegenerative disease of the central nervous system (CNS) that affects young individuals, with significant cost to society. In the past 10 years, tremendous progress has been made in identifying the roles of multiple immune cell gene variants in disease susceptibility and new disease-modifying therapies (DMTs) to regulate immune cell activity [1]. The current, available DMTs decrease the number of relapses but do not stop the progression of the disease, which eventually leads to brain atrophy and disability [2]. Little progress has been made in the prevention and management of such disease progression in MS, because the mechanisms underlying the disease pathogenesis are still unknown. Several hypotheses have been proposed, including (a) persistently activated microglia [3], (b) formation of inflammatory follicles within the meninges, (c) the potential intrinsic selective vulnerability of the neurons and axons to different injuries [4], and (d) the absence of myelin repair that contributes to continuous axonal loss [5]. All of these will result in neuronal damage and progressive neurodegeneration [2]. The mechanisms underlying the lack of myelin repair and their contributions to the ongoing progression of MS are unknown; however, work in animal models of MS has suggested that cells with the capacity for repair, such as oligodendrocyte progenitors and other neural progenitor populations, are targeted by long-term chronic inflammation [6, 7].

Owing to the inaccessibility of primary tissues, such as the brain, and their limited growth in vitro, transgenic mice and immortalized neuronal cell lines have been valuable tools for studying the pathogenesis of neurological diseases. However, animal models do not accurately phenocopy disease pathology and fail to recapitulate consistent cellular and molecular phenotypes relevant to human diseases. For instance, multiple compounds that inhibited neurodegeneration in mouse models have failed to do so in human clinical trials, probably because of interspecies differences [8]. Our lack of understanding and progress in these aspects of the neurodegenerative component of MS remains a significant problem, because patients' neurodegeneration and brain

atrophy continue to progress. Therefore, strategies and tools aimed at understanding these critical aspects of the disease will be welcome.

Recent progress in somatic cell reprogramming and induced pluripotent stem cell (iPSC) technology has allowed the generation of disease target cells in a patient-specific setting. iPSCs are pluripotent stem cells that can be generated from a variety of somatic cells by the forced expression of transcription factors (Oct4, Sox2, Klf4, and c-Myc) involved in the maintenance of pluripotency in embryonic stem cells (ESCs) [9, 10]. Notably, iPSCs can be differentiated into the affected cell type of interest in vitro using the same protocols developed for ESCs, and they can provide a potentially unlimited renewable source of patient-specific cells for disease modeling and drug screening and novel cell replacement therapy approaches [11–13] (Figure 1). To date, human iPSCs (hiPSCs) have been generated from healthy individuals and from those affected by a variety of diseases, including several neurological disorders [14]. Reprogramming cells from patients with Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis has contributed to our understanding of these diseases and suggested new therapeutic targets [13, 15–18]. Therefore, great hope exists that reprogramming somatic cells from patients with MS to generate neurons, astrocytes, and oligodendrocytes in a patient- and disease subtype-specific setting will provide researchers with a unique biological tool to understand the mechanisms of the disease, especially relating to therapy, cellular models, or drug screening for neurodegeneration and repair [19]. In animal models of MS, stem cells have already emerged as a powerful strategy to ameliorate the disease, and mesenchymal stem cells (MSCs) are being investigated in human clinical trials [20, 21]. However, the application of stem cells to model a complex neurological disease such as MS still presents technical challenges. We have reviewed the current state and promise of using hiPSCs derived from patients with MS and human inducible neurons (hiNs), human inducible oligodendrocyte progenitor cells (hiOPCs), or other stem cell platforms such as immunoregulatory neural stem cells to understand and model critical aspects of the cellular and molecular pathology of MS.

Modeling Neurological Diseases With iPSCs

In recent years, from the efforts of the genetics consortia and multiple laboratory collaborations, we have made progress in the genetics and molecular basis of several neurological diseases, including PD, AD, and HD [1, 22, 23]. These innovations, coupled with the ability to generate brain cells from human skin fibroblasts, have led to new strategies in patient-oriented research [13, 24]. iPSCs from these patients retain their genetic vulnerability traits and represent a novel and important setting in which to examine susceptibility to neurodegeneration and aging. Monogenic forms of diseases with full penetrance are more likely to display robust cell-autonomous cell defects in stem cell-based modeling systems. Thus, one of the foundations of in vitro stem cell modeling of neurological diseases has been to select monogenic forms of diseases with a clear genetic and molecular dysfunction. The vast majority of diseases in which iPSCs have been generated thus far have fit this criterion. Of approximately 20 different neurological diseases in which iPSCs have been generated as proof-of principle, only a few have been sporadic (Table 1) [25]. These efforts have been facilitated by selecting diseases in which the functional consequences of the somatic mutations have been validated in mouse models [23, 26].

Mutation-defined iPSCs can generate models of neurodevelopmental disorders, and their respective phenotypes have been used to test novel therapies. Cortical neuronal precursor cells developed from iPSCs derived from patients with Timothy syndrome (TS), a neurodevelopmental disorder caused by a missense mutation in the *CACNA1C* gene, displayed defects in calcium signaling, activity-dependent gene expression, and increased production of norepinephrine and dopamine. These phenotypes were reversed when treated with roscovitine, a cyclin-dependent kinase inhibitor and an atypical L-type channel blocker [27]

Similarly, in Rett syndrome (RTT), caused by mutations in *MECP2* [28], mouse models have suggested a non-cell-autonomous role for astrocytes in RTT pathogenesis. iPSC-derived astroglial progenitors from patients with RTT showed adverse effects on the morphology and function of wild-type neurons, independent of any intrinsic neuronal deficits, confirming a previously suspected non-cell-autonomous role suggested for glia in RTT disease pathology. Insulin-like growth factor 1 was found to rescue the neuronal deficits caused by mutant RTT astrocytes [28]. Therefore, the use of iPSC-derived models of patients with RTT and TS recapitulate key features of disease and substantiate the feasibility of using hiPSCs as tools for studying multigenic neurological diseases for both discovery and potential treatments in which intrinsic neurodevelopmental components and glial cells could affect disease pathology.

In a similar fashion, the use of mutation-defined iPSCs can generate human cell models of neurodegeneration and new cellular and molecular phenotypes [23]. Several studies have pursued iPSC-based modeling for AD associated with familial mutations in presenilin: *PSEN-1*, *PSEN-2*, or *APP*. Such cell lines can be used to understand the contributions of different molecular pathways in the pathological cascade of a disease. Using iPSCs from patients with AD, recent studies have shown that different mutations can lead to the same neurological phenotypes by molecular mechanisms affecting distinct molecular networks [26].

A promising and exciting direction is the investigation of iPSCs from patients with nonfamilial forms of AD carrying common genetic variants such as *APOE* and *SORLA/SORL1* loci that significantly affect sporadic AD risk [29]. This approach will help define additional neuronal phenotypes such as synaptic, axonal functioning, and signaling pathways that dampen oxidative stress in vitro. Using nonfamilial AD-derived iPSCs, investigators have shown that iPSCs-derived neurons from patients with sporadic AD exhibit similar phenotypes to neurons from familial forms, suggesting common pathogenetic mechanisms [30]. Genetic alterations, perhaps a copy number variation not identifiable using current genome-wide association study (GWAS) strategies, appear to affect the cellular function in these sporadic forms in a manner similar to AD-causing mutations [30]. This has important implications in a disease such as MS, in which no genetic variants autonomously affecting neuronal function have been identified thus far, and emphasizes the power of disease modeling to reveal strong neuronal pathological phenotypes in patient-derived neurons in vitro.

Modeling MS With iPSCs

In contrast to AD, PD and other neurodegenerative diseases in which a percentage of patients have defined somatic mutations,

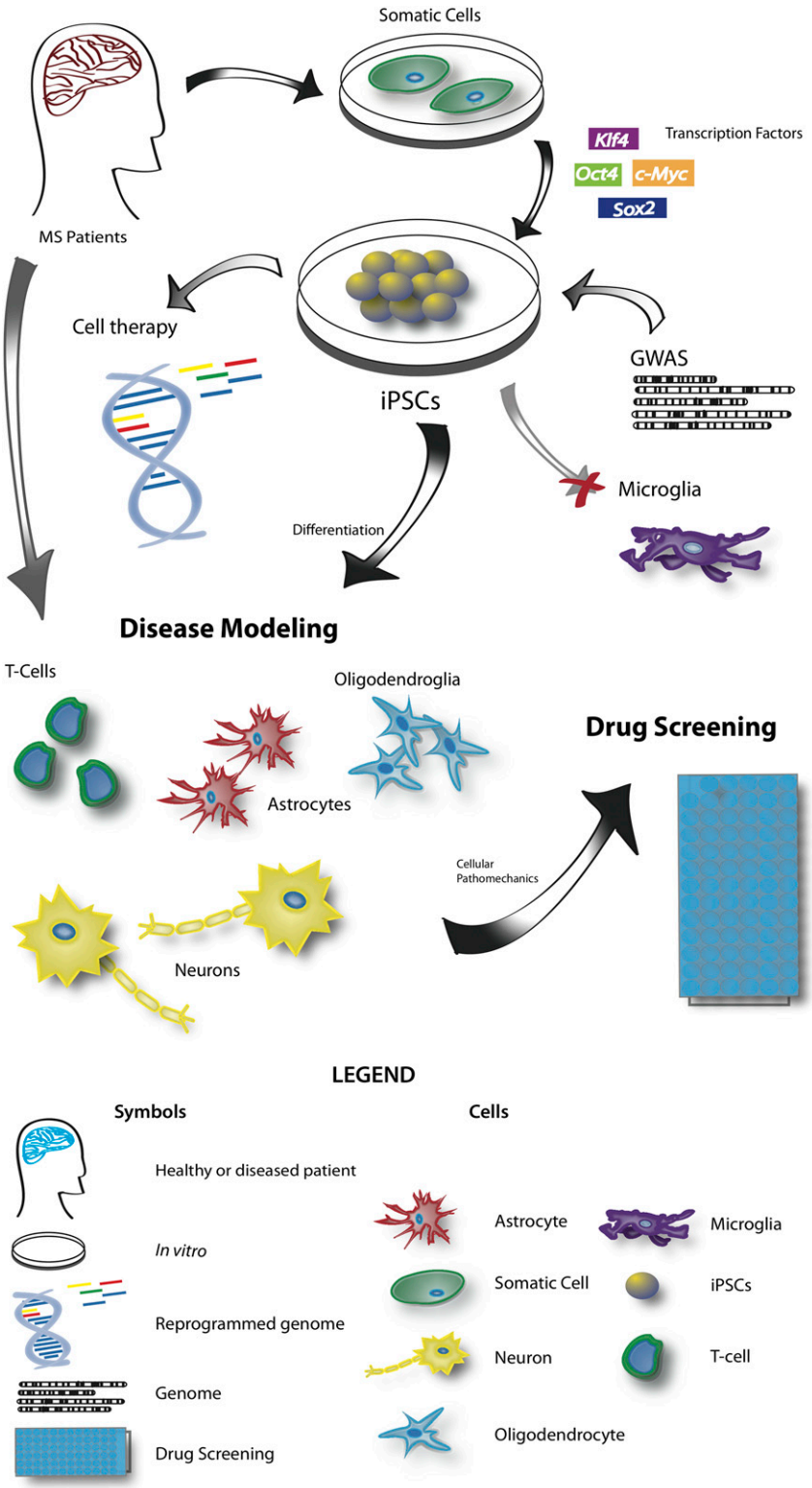

Figure 1. Human iPSC graphic schematically highlighting the usage and directives of iPSCs, especially pertaining to disease modeling and drug screening of MS using patient-derived cells. Abbreviations: GWAS, genome-wide association study; MS, multiple sclerosis; iPSC, induced pluripotent stem cell.

MS is a complex disorder not caused by single genetic mutations but associated with multiple susceptibility genes in immune cells [1]. Our current understanding is that modest contributions of genetic variants in the genes associated with antigen-presenting cells and T-cell function are associated with susceptibility to MS [1]. One of the most significant issues in MS therapy is that despite current treatment, patients continue to accumulate CNS damage and neurological disability. The assumption has been that MS

Table 1. Summary of iPSCs derived from neurological diseases

Disease	Genetic defects/variants
Monogenic neurological disease	
Huntington's disease	CAG expansion in *HTT* gene
Familial dysautonomia	IKBKAP
Spinal muscular atrophy	SMN1
Machado-Joseph disease	CAG expansion in ATXN3
Multigenic neurological disease	
Alzheimer's disease	PSEN1, PSEN-2, APP duplication
Parkinson's disease	LRRK2, PINK1, SNCA, Parkin
Amyotrophic lateral sclerosis	SOD1, VAPB, TDP43
Rett syndrome	MeCP2, CDKL5
Down syndrome	Trisomy 21
Timothy syndrome	CACNA1C
Adrenoleukodystrophy	ABCD1
Sporadic neurological disease	
Schizophrenia	Multiple CNV
Sporadic Alzheimer's disease	Multiple
Sporadic Parkinson's disease	Multiple
Multiple sclerosis	Mainly T-cell variants

is a primary autoimmune disorder in which its long-term progression is related to the activity of T cells. Although the role of T cells in initiating damage is well accepted, recent work by several laboratories worldwide have identified a neurodegenerative component for MS [2, 3, 31]. It is still unclear to what extent the interaction between cell-autonomous mechanisms in neural cells (neurons, oligodendrocytes, and astrocytes) and the immune dysfunction contributes to the observed neurodegeneration. To address these questions, patient-specific neural cells carrying the susceptibility genetic traits are required. However, in MS, no robust neuronal genetic variants have been established, although recent work of the genetic variants of the glutamate levels has shown potential implications in neuronal damage [4]. However, more neuronal and oligodendrocyte variants need to be identified [32]. An important task is to define the aspects of the disease that can be modeled using iPSCs and the goals of generating patient-derived brain cells in MS compared with other neurological diseases, for which much of the work relies on using patient samples with pathogenic mutations. In addition, the mechanism of progression of MS and neurodegeneration might involve other cells, including OPCs [33] and astrocytes [34]; therefore, the generation of these cells from patients could be critical to understanding the participation of such cells in the mechanism of progression. The generation of a variety of patient-derived neural cells would help in the identification of the specific defects or variants that might be associated with disease neurodegeneration and lack of repair [2]. Recently, neurons and oligodendrocytes from patients with MS have been generated as a proof-of-principle; however, no alterations in their intrinsic biology has yet been reported [35, 36].

Several obstacles exist to directly exploring the pathophysiology of MS with human tissue. With the outstanding questions of the physiopathology of the disease, we could consider several modeling goals and hurdles (Table 2). Efforts are needed to use brain cells relevant to the pathology of MS, such as the oligodendrocytes and neurons that are targets of the disease process. However, brain cells are not readily available unless obtained directly by biopsy, which is rarely clinically justified. In addition, oligodendrocytes and neurons are postmitotic cells requiring their precursors. Therefore, the development of neural cells from patients with MS carrying disease-specific genetic variants to study the intrinsic alterations that affect the biology of neurons and oligodendrocytes would be highly desirable. An important question that arises is whether MS patient-derived neurons have an intrinsic genetic defect. In other sporadic diseases, such as schizophrenia, iPSC-derived neurons have shown that synaptic connectivity is reduced compared with that of normal controls [37]. Therefore, using iPSC-derived neurons from patients with MS, abnormalities in synaptic or axonal function or other neuronal and oligodendrocytic cellular phenotypes could be identified. These phenotypes could help in the identification of the variants associated with alterations and the vulnerability of neurons to injury. However, these efforts will require extensive collaboration among laboratories and consortia because of the high number of required cell lines. One key goal is to establish a repository of human cells, including induced oligodendrocyte progenitors cells (iOPCs) and hiNs, to advance the search of new cellular phenotypes and the screening for compounds to halt neurodegeneration. However, current protocols to generate OPCs and specific neuronal subtypes relevant to the disease from iPSCs are lengthy and still require a great amount of technical expertise [38]. These efforts should be accompanied by GWAS strategies to find the networks of genes associated with regeneration and neuronal survival that are dysregulated in these cells. A more attainable goal in the short term would be to use cells from patients with monogenic white matter disorders that can be used to illuminate certain aspects of the vulnerability of oligodendrocytes to damage and myelination in vitro. Recently, mouse embryonic and human fibroblasts were programmed into functional iOPCs that showed the capacity to remyelinate both in vitro and in vivo, although a direct comparison of iOPCs from patients and controls is still lacking [38, 39]. The study of the neuroprotection of neurons and oligodendrocytes, two major targets of T cells, will benefit from these new models, because we could study the effects of T cells in the molecular mechanisms that lead to neuron and oligodendrocyte dysfunction. Another important goal will be modeling the vulnerability of neurons to stressors relevant in MS pathology, such as oxidative stress, glutamate toxicity, and inflammatory cytokines [4, 40].

In contrast to postmitotic neural cells, T cells are easily available from patients with MS, and it is not necessary to recreate them by reprogramming. However, the generation of T and B cells from patient-specific iPSCs would limit the amount of blood needed. Microglia, which play a key role in neurodegeneration and repair in MS, have not yet been generated from human pluripotent stem cells. However, new data have suggested they can be generated from human monocytes, replicating key features of human microglia [41]. The implementation of these strategies will facilitate coculture systems to model the initial neurotoxic versus reparative interactions of microglia and T cells with neural cells. These advances are essential to determine the human disease mechanisms and find new molecular therapies using high throughput assays.

Table 2. Goals and hurdles of modeling neurological disease with iPSCs

Goals
GWAS strategies coupled with iPSCs to find networks of genes associated with regeneration and neuronal survival (endophenotypes)
Model the effects of T cells and inducible neuron/oligodendrocyte alteration
Develop isogenic controls using the same patient cell line in which the mutation is "corrected," allowing for genotype-phenotype functional correlation studies
Coculture systems to model microglial and T cell initial neurotoxic vs. reparative interactions with inducible neural cells
Use iPSCs derived from patients with MS or MS-like monogenic disorders to identify novel neuronal or oligodendrocytic phenotypes
Model the vulnerability of inducible neurons to MS stressors (oxidative, glutamate toxicity, inflammatory cytokines)
Establish a repository of human cells, iOPCs, and hiNs to search for novel cellular phenotypes and screen for compounds to halt neurodegeneration
Improve personalized therapy in MS by administering medications found in cellular assays based on cellular signatures
Hurdles
No neuronal genetic variants identified
Skin fibroblasts from older patients might have low reprogrammed efficacy
Irreversible transcriptional depression of genes on the X chromosome of female-generated iPSCs in vitro
Lack of genetically matched nondiseased controls
Defects produced by reprogramming in vitro could overshadow an intrinsic vulnerability to dysfunction in MS-derived neurons
Microglia not yet generated from human iPSCs
iPSCs kept in vitro for extended periods could have genomic instability
Genetic variance of immune cell function could be associated with neurodegenerative susceptibility
MS-derived cells might not provide strong disease phenotypes in vitro

Abbreviations: GWAS, genome-wide association study; hiNs, human inducible neurons; iOPCs, inducible oligodendrocyte progenitor cells; MS, multiple sclerosis.

Technical Considerations for Modeling MS With iPSCs

iPSCs have shown the potential to generate insights into disease mechanisms and open new opportunities for clinical intervention through the identification of novel mechanisms and pharmacological targets. However, previous experience with iPSC-based disease modeling has led to some important technical considerations that pose additional challenges that are magnified by the complexity of MS. The possibility of select ing donors with specific genotypes will provide opportunities to understand the mechanisms associated with the presence of these genetic variants. However, cells with genetic variants, rather than somatic mutations or microdeletions, might not generate a strong disease phenotype in vitro. Therefore, a critical unanswered question is whether many of the functional variants or single nucleotide polymorphisms (SNPs) important for neuronal phenotypes could be masked by the defects produced by the reprogramming process itself and the heterogeneity among different pluripotent stem cell lines. In contrast, the cells generated from patients with frank deletions and mutations will have a greater effect on the underlying genetic network that could overcome the genomic changes induced by reprogramming. These considerations are critical to validate the functional role of copy number variants with a modest effect on neurodegeneration in the dish.

Another critical issue is the minimum number of clones required to detect biologically relevant phenotypes. Even in circumstances in which patients have a clear genetic abnormality, multiple patients and controls are required to generate enough cell lines to obtain meaningful data for functional validation beyond proof-of-principle studies [36]. The source of cells is another important concern, because, in some studies, human skin fibroblasts from older individuals have shown very low efficiency. However, others have found acceptable efficiency, although the new reprogramming techniques are more effective [42, 43]. This would be particularly important when cells from patients with primary or secondary progressive MS are used, because these patients have usually been diagnosed later in life. The reason for the lower efficiency of reprogramming with cells from older patients is unknown. These challenges are significant to all genetic and sporadic neurodegenerative diseases, including MS.

The goal of generating disease-specific hiPSCs is to identify novel cell autonomous or non-cell-autonomous processes and pathways that are difficult to investigate using autopsy and biopsy tissues. However, one important issue is the lack of genetically matched, nondiseased controls, making attribution of the observed phenotypes to the disease-causing mutation difficult. This confounding issue must be solved, because differences in the genetic background in humans and the variations among cell types could influence data interpretation. To provide improved and more rigorous controls, investigators have generated isogenic controls (i.e., using the same patient cell line but with the mutation "corrected"). This approach provides the most rigorous control for experiments in which an effect of a genetic mutation needs to be verified. These have been achieved in human iPSCs, using powerful tools for the manipulation of the human genome, including zinc fingers and transcription activator-like effectors, nucleases, and the clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9 system [44, 45]. Using such approaches, disease-associated genetic variants can be replaced with wild-type (WT) constructs by homologous recombination in patient-derived cell lines. Alternatively, it is also possible to insert candidate disease-associated genetic variants into the endogenous WT locus in control lines by homologous recombination

or knockout endogenous genes of interest [26, 46]. Importantly, the comparison between patient and isogenic gene-corrected cell lines allows for genotype-phenotype functional correlation studies [18, 47].

Using these controls and integrative functional genetics, it will be possible to study not only the major common cellular phenotypes, such as cell death, cell differentiation, and cell growth, but also novel clinical relevant phenotypes and endophenotypes. Integrative genetics and cellular modeling could unveil new cellular and molecular phenotypes from patient-derived neurons or oligodendrocytes, which could be used to identify novel biomarkers and therapeutic interventions. Notably, it has been shown that in sporadic neurological diseases, alterations occur in the molecular networks in the absence of somatic mutations [48]. For example, emerging data using tissues from the brains of patients with AD have indicated that in sporadic cases, dysfunctional molecular networks are associated with CNS injury [48]. In addition, studies investigating expression quantitative trait loci (eQTL) are identifying molecular phenotypes or endophenotypes in AD that could be amenable to pharmacological targeting. These findings could be explained by subtle epigenetic or copy number alterations in genes with a central role in the biological network targeted by the disease [49]. Thus, the dysregulation of such biological networks in vivo could be investigated in vitro with patient-derived cells to find relevant targets for therapeutic interventions [48]. Future studies are needed to find new molecular phenotypes and eQTLs in MS brain tissue that can be replicated and modeled in the dish with iPSCs. The holistic goal is to illuminate novel mechanisms by implementing these tools to study a sporadic neurological disease such as MS [48].

The assumption that the reprogrammed cells are identical to the diseased neurons in situ or even to the original fibroblasts has been challenged by several studies that have shown that the genome and epigenome of reprogrammed cells differ from those of the parental cells [50, 51]. Pluripotent stem cells are kept in vitro for extended periods, which can also introduce genomic instability [51]. Additional issues concern the inherent heterogeneity and functional variability among iPSC lines and within different clones from the same individual. Additionally, female iPSCs with time in culture undergo "erosion'' of the X chromosome, and this inactivation leads to irreversible transcriptional derepression of genes in the inactive X chromosome [52]. This erosion has been demonstrated to have a significant effect in the modeling of X-linked neurodegenerative diseases. The effect of these alterations on the investigation of disease mechanisms in cells generated from patients with neurological diseases, including those with MS, remains unclear [36, 52, 36]. Perhaps the use of iPSCs derived from family trios and twins with MS could reduce the variability of these cultures owing to their shared genetic material.

Use of iPSCs to Correlate MS Phenotype-Genotypes and Drug Discovery

The goal of in vitro disease modeling with iPSCs is to recapitulate key aspects of the disease and gain a better understanding of the cellular and molecular mechanisms underlying disease pathology and perform drug screening. The observed MS-derived neural cell phenotypes should be robust and reproducible. Therefore, our evaluation of cellular phenotypes in human cells in vitro should be improved. The in vitro evaluation and optimization of a number of phenotypes needs to improve, including synaptic plasticity, axonal growth, myelination, and electrophysiological function of the neurons, astrocytes, and OPCs, in addition to changes in gene expression. All will be important for the detection of new disease-related phenotypes or endophenotypes in MS, just as they have been for other neurological disorders [13, 23, 24, 26, 37]. The investigation of hiOPCs derived from monogenic disorders in which myelination and oligodendroglia survival is affected, such as from patients with Pelizaeus-Merzbacher disease with PLP1 mutations, will allow gain- and loss-of-function studies in hiOPCs and, eventually, shed light on MS pathophysiology [53]. Next, the phenotypes identified in such genetically defined cohorts can be investigated in oligodendrocytes isolated from sporadic or familial cases of MS. In addition, robust assays are needed that can show disease-relevant phenotypes easily scalable to high-throughput drug screening. Recently, it has been shown that benzatropine, an antimuscarinic compound, identified using stem cell-based high-throughput drug screening, improved the outcomes in a model of MS by enhancing OPC function and remyelination in vitro and in vivo [54]. These findings were corroborated using a modified OPC-based assay termed "binary indicant for myelination using micropillar arrays" that showed that two antimuscarinic agents, benzatropine and clemastine, promoted remyelination in vitro and in vivo [55]. Therefore, optimization of automated multiwall-format assays using stem cells and OPC arrays is an important goal for MS stem cell modeling and the search of novel reparative compounds. These medications can now be tested in clinical trials in patients with MS. Finally, the use of a issue-engineered approach from hiPSCs that simulates three-dimensional CNS structures has been beneficial, because they recapitulate important processes in development and disease [56].

It is crucial to find the genetic variants that are important for the neuronal dysfunction to be investigated in vitro with iPSCs. However, to find variants using GWAS, we need a robust neuronal phenotype that can be identified through clinical features, imaging, or serum biomarkers. In the case of MS, our current imaging biomarkers for neurodegeneration and repair are limited. The reverse strategy, identifying neuronal variants by first generating numerous neuronal lines from patients to find new cellular phenotypes in vitro and then performing GWASs would require enormous resources. The validation of SNP variants in neurons, astrocytes, and oligodendrocytes will require sharing data and consortia and collaboration among laboratories.

Modeling Neuroprotection in MS

To make neurons more resilient to injury, we need to create targeted neuroprotective strategies. iPSCs can also be used to predict vulnerability to toxicity in the cells that are a part of the MS pathology (i.e., neurons, oligodendrocytes, and astrocytes). This approach can be used to find neuroprotective compounds. In addition, the response of stem cells from individualized patients, in which a desired response is matched to a variant or genomic signature, can be combined. Cellular assays based on these individual cellular signatures could lead to personalized therapy, especially if Food and Drug Administration-approved drugs can be repurposed to rapidly detect new targets. These efforts could lead to a new strategy by which patients at a relapse or evidence

of sustained inflammation and progressive disease can be treated with the established DMTs and novel neuroprotective compounds identified in stem cell-based assays with different selectivity to neurons and oligodendrocytes, such as has been shown recently in mice [54].

Modeling Immune Cells/Neuronal Interactions

In MS, extrinsic factors (non-cell-autonomous mechanisms) play a crucial role in neuronal and oligodendroglia pathology. Injury to neurons can be conferred by immunological responses of infiltrating macrophages, T cells, and neighboring microglia [50, 57]. In vitro coculture approaches offer a reductionist model to address the role of T cells and microglia. Our knowledge of the pathogenic mechanism of T cells in humans is limited, and the proposed mechanisms have mostly been based on experiments in mice. iPSC technology could help model such non-cell-autonomous mechanisms and early interaction of human immune cells, neurons, and oligodendrocytes occurring in human disease. Using inducible neurons from patients with MS and control individuals, it might be possible to model the initial interaction of T or B cells with neurons or oligodendroglia from the same patient. In mouse models of MS, it is known that T cells can induce fluctuation in neuronal intracellular calcium concentration and damage of axons in vivo [58]. Therefore, cocultures of human T cells obtained from the blood and axons from neurons derived from the same patient with MS can be studied to find compounds that protect the axons from the deleterious effects of T cells from individual patients. Moreover, such a system can be used to dissect the beneficial versus neurotoxic effects of human microglia on neurons and oligodendrocytes [6].

It is unknown whether all the MS autoreactive T cells have the same capacity of inducing neuronal damage; therefore, the genetic variation of T cells involved in neuronal damage can be explored by selecting patients with specific T-cell variants that confer vulnerability to excitotoxicity or inflammation. This would improve our understanding of "second hits" in neurodegeneration in MS. In addition, T cells and microglia can affect neural stem cell niches [6, 7]; therefore, the development of in vitro models of neural stem cell niches with iPSCs could improve our understanding of the non-cell-autonomous effects of the disease on neural progenitor cells.

Finally, iPSC-derived immunoregulatory neural stem cells have emerged as a possible therapeutic strategy in mice [59]. However, clinical trials addressing the safety and efficacy of human stem cells are still in the initial phases. The therapeutic benefits of stem cells are likely related to the bystander immune and neuromodulatory effects of their secreted compounds such as leukemia inhibitory factor, hepatocyte growth factor [59, 60], and other bioactive molecules in the stem cells. Therefore, iPSCs offer the opportunity to investigate the beneficial effects of these bioactive compounds using patient-derived neurons, oligodendrocytes, and axons in vitro.

Future Directions

Several difficulties remain in modeling a complex disorder such as MS with iPSCs. One question that remains is how many cell lines are necessary to establish phenotypes in MS patient-derived cells. Moreover, better methods are needed to reprogram cells that reflect the in vivo behavior of diseased neural cells. Furthermore, we must improve the measurement of functional molecular and cellular phenotypes in vitro that are relevant to the disease process. Efforts must be made to obtain cohorts of patients and controls of sufficient size for statistical analysis. Finally, we should use genetic or biochemical complementation to link a genetic variant to the phenotype by creation of isogenic "rescued" neural cells to provide the most rigorous controls.

A paramount goal is to generate repositories of MS-derived neural cells to identify cell-specific genetic variants. We suggest, therefore, that a consortium should be established to generate the large number of cells and data needed. These cells should be deposited in well-cataloged biorepositories and made available to all investigators.

Conclusion

In the present review, we delineated the potential for stem cell technologies, especially patient-derived stem cells, to inform fundamental aspects of MS, with a particular emphasis on the neurodegenerative component to oligodendrocytes, neurons, and neural progenitors. Despite the many issues that still need to be solved, stem cell-based assays, including patient-derived cells and modeling, have the potential to become an important tool in the study of the complexity of inflammatory-mediated neurodegeneration and repair, leading to better translation and drug discoveries to help patients affected by MS.

Author Contributions

J.I.: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, study supervision; J.C.O.: acquisition of data, analysis and interpretation of data, critical revision of the manuscript for important intellectual content; M.D., D.P., F.W., J.A.N., and A.L.B.: acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content; M.K.R.: drafting of the manuscript, critical revision of the manuscript for important intellectual content; M.C.: analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content; K.M.: drafting of the manuscript, critical revision of the manuscript for important intellectual content.

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Concise Review: Modeling Multiple Sclerosis With Stem Cell Biological Platforms: Toward Functional Validation of Cellular and Molecular Phenotypes in Inflammation-Induced Neurodegeneration

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