Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

ELSEVIER

Using stem cells and iPS cells to discover new treatments for Parkinson's disease

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ARTICLE INFO

SUMMARY

Fetal cell transplantation can improve the symptoms of Parkinson's disease (PD) patients for more than a decade. In some patients, alpha-synuclein aggregates and Lewy bodies have been observed in the transplanted neurons without functional significance. Recently stem cells have emerged as an ethically acceptable source of cells for transplantation but, importantly, the type of stem cell matters. While the lineage restriction of adult neural stem cells limits their clinical applicability for patients with PD, human pluripotent stem cells provide an opportunity to replace specific types of degenerating neurons. Now, cellular reprogramming technology can provide patient-specific neurons for neural transplantation and problems with cell fate specification and safety are resolving. Induced pluripotent stem (iPS) cell-derived neurons are also a unique tool for interpreting the genetic basis for an individual's risk of developing PD into clinically meaningful information. For example, clinical trials for neuroprotective molecules need to be tested in presymptomatic individuals when the neurons can still be protected. Patient-specific neural cells can also be used to identify an individual's responsiveness to drugs and to understand the mechanisms of the disease. Along these avenues of investigation, stem cells are enabling research for new treatments in PD.

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1. Introduction

Within the last decade, considerable biotechnological innovation has led to the use of stem cells in Parkinson's disease (PD) research in topics ranging from cell therapy to individualized disease modeling. In this review, we discuss the state of the field and future directions of using stem cells for PD research.

2. Using stem cells to generate ventral midbrain dopaminergic neurons for cell therapy

The transplantation of human fetal ventral midbrain (VM) type dopaminergic (DA) neurons into PD patients can provide safe, long-term therapeutic benefits [1,2]. While alpha-synuclein aggregates and Lewy bodies have been demonstrated in a subpopulation of the grafted VM DA neurons in some of the PD patients that received fetal cell transplantation [3,4], the functional significance of the observations for the patients and their families remains unclear [5,6]. In spite of these controversial findings, translational research has continued to focus on using stem cells to derive a therapeutically relevant and ethically acceptable population of VM DA neurons to treat patients. As the research has continued to develop, several sources of stem cells have been examined for their ability to improve behavioral measures in rodent models of

PD. Early studies of adult neurogenesis raised the possibility that endogenous stem cells committed to the neural lineage and found in the patients' brain could be recruited from the subventricular zone and differentiate into DA neurons [7]. While numerous studies have shown that adult neural stems reside beneath the lining of the lateral ventricles and their cellular progeny can proliferate and migrate into the target synaptic field that is denervated in rodent models of PD, the subsequent differentiation into therapeutically relevant DA neurons has not been realized [8,9]. Subsequently, lineage restriction has been shown to limit the ability of adult neural stem cells to differentiate into VM DA neurons and can only be removed by cellular reprogramming [10,11].

Alternatively, embryonic stem (ES) cells have received considerable attention as a source of VM DA neurons given their potential to differentiate into any cell type of the body. Beginning with the derivation of mouse embryonic carcinoma and ES cell lines, several laboratories have focused on using these cells as a source of DA neurons. Initially, the low dose transplantation of mouse ES cells in rodent models of PD led to default differentiation into neural cells, including DA neurons [12]. With improvements in cell culture conditions, differentiation protocols following developmental principles, and innovative cell type reporters and purification strategies, mouse ES cell-derived neural cell populations enriched for VM DA neurons have been safely transplanted into rodent models of PD, improving behavioral responses [13]. These approaches have been applied to mouse induced pluripotent stem (iPS) cells with similar results [14]. The translation of the studies using mouse ES and iPS cells to their

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human equivalents has been technically demanding but recent technological advances provide safe populations of neural cells that include VM DA neurons for functional integration [15–17] and the yields of therapeutically relevant VM DA neurons from human ES/iPS cells are improving [18]. As a source of transplantable human VM DA neurons, ES/iPS cell-based therapies for PD are moving towards clinical trials.

3. Parkinson's disease modeling using patient-specific iPS cells

There are times when advancing technologies provide opportunities beyond the typical stepwise approaches that are necessary to establish scientific findings by standard methods. One such example is the use of patient-specific iPS cells to make new assays possible for research on neurodegenerative diseases.

Studies of families with an unusually high incidence of PD combined with genome sequencing technology can identify individuals who are at risk for developing PD [19]. However, we still do not know how to translate these findings into the clinic. As a first step, human cell models with an authentic risk load, such as patient-specific iPS cells have been used to model several diseases. However in contrast to PD, these studies have targeted monogenic diseases that are developmental in nature. Animal models, including transgenics or direct neuronal toxicity have provided useful clues to PD pathogenesis. Nevertheless, none of these systems are of a human cellular origin or context and do not adequately simulate all of the factors that descend on the cell prior to degeneration. Even the genetic animal models tend to have a particular PD version or pathology, not necessarily reflecting the degeneration seen in human cases of the same genetic hereditary marker. To compound this lack of adequate modeling, many of the degenerative outcome measures have focused on neuronal cell death, rather than cellular and synaptic dysfunction or important molecular prodromal or pre-degenerative changes. The pre-degenerative cellular changes are likely the most important processes in time spans for which treatments would have a neuroprotective impact and provide functional benefits. With this perspective in mind, we believe that the availability of human patient cells made broadly possible by very recent innovations in iPS stem cell biology provide new avenues for testing and evaluating degenerative changes, and also eventually for testing strategies against disease. The well-characterized genetic risk of familial PD is a reasonable starting point for determining PD iPS derived neural phenotypes in a broad population of differentiated cell types (Fig. 1A).

Patient-specific iPS cells can potentially provide any cell type of the body, including the vulnerable and resistant neural cell types that are important for modeling age-, cell- and tissue-dependent neurodegenerative diseases such as PD. Therefore, specific neural cell subtypes differentiated from iPS cells are likely to be a powerful tool for evaluating the genetic contributions to disease mechanisms, the hierarchy of cellular vulnerability and potential therapeutics.

Cell type specific vulnerability is exemplified by PD, in which there is relative vulnerability even among neighboring midbrain neuronal populations releasing the same neurotransmitter, DA. On a neural systems scale, selected regional neuronal populations [norepinephrine (NE), serotonin (5-HT), acetylcholine (ACh), gut and PNS neurons] display distinct high or low risks for PD-like pathology. Regardless of specific etiology, including aging, DA neurons in the substantia nigra (A9) are considerably more vulnerable than DA neurons in the immediately adjacent ventral tegmental area (A10) both in vitro and in vivo. A similar pattern of differential vulnerability is observed in rodent and primate models of PD, indicating that such differential vulnerability between A9 and A10 DA neuronal populations is conserved between species.
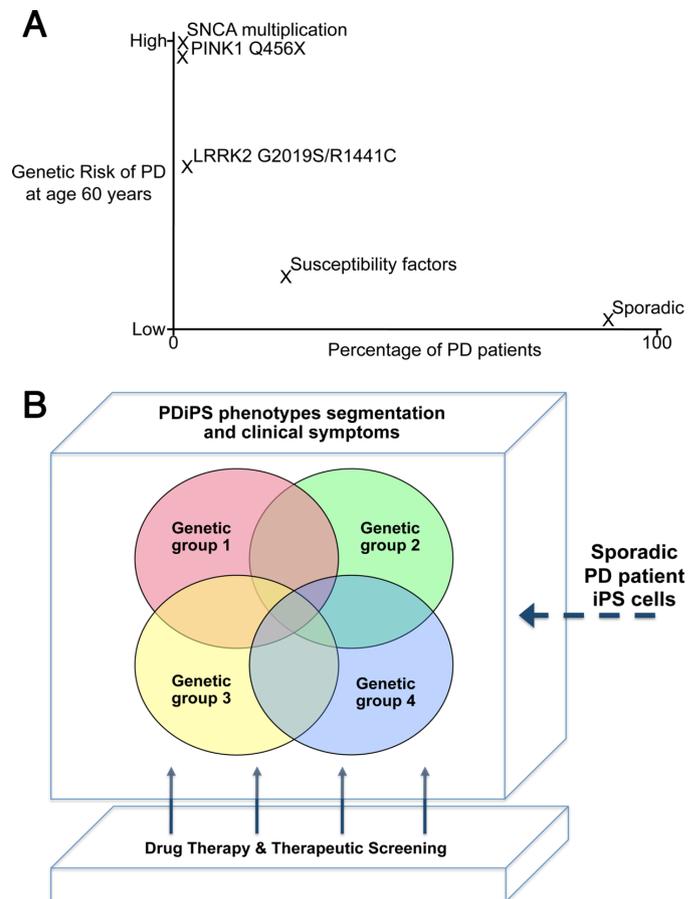

Fig. 1. Using PD iPS phenotypes to individualize medicine for Parkinson's disease patients and at-risk individuals. (A) The genetic contribution to an individual's risk of developing PD is generally very low. 85–90% of patients with sporadic PD have little or no genetic risk factors but they share clinical symptoms with the rare individuals with familial forms of PD. While the genetics of familial PD has driven basic and translational research, genome wide association studies are beginning to identify an intermediate level of genetic risk (susceptibility factors). The PD iPS Cell Line Research Consortium has generated PD iPS cells from such rare individuals with known disease-causing mutations (genetic PD iPS cells) to create a unique platform for coanalyzing PD associated phenotypes caused by second hits of cellular stress. By grouping the genetic PD iPS cell lines according to shared phenotypes and the clinical symptoms of the donor patients, we think that we can translate this platform to screen drugs that modify specific phenotypes and thus identify responsive patient and at-risk cohorts. Furthermore, we can use the axes of genetic PD iPS neural cell vulnerability in an effort to examine how individual sporadic patient iPS lines may align with genetic PD iPS groups and predict drug responsiveness.

Furthermore, rodent A9 and A10 DA neurons have distinct gene expression profiles despite their many similarities. Such inherent baseline gene expression differences create biochemical identities that set different thresholds of vulnerability to both rare and common pathophysiological processes. Genomic analyses concentrate on comparing normal and disease states and the resultant analyses highlight major differences in gene expression profiles of cell death pathways. These approaches provide valuable scientific information but tend to be limited because they do not take into account the normal physiological differences between vulnerable and resistant neurons.

To use these new PD genetic cellular tools to examine pathogenesis, a second set of molecular stressors is likely to be needed that force the cells to express phenotypes in a disease-relevant context (Fig. 1B). The same reasoning applies to the future opportunity to use genetic PDiPS cells to better define overlapping and different sets of PD patient populations; and make drug discovery relevant to the patients' own cellular responses and disease type (Fig. 1B).

With new iPS technology, patient-specific neuronal subtypes have become a realistic experimental tool. However, generating homogeneous preparations of neuron types for mechanistic assays is a real challenge and requires innovation. Typical iPS cell differentiation protocols generate a broad range of asynchronous neural cell types that will need to be depleted prior to assays of cell type-specific neurodegeneration. Such protocols for purifying cell types differentiated from human iPS cells include cell surface markers and genetically encoded fluorescent reporters [17,20]. Furthermore, new technologies can address the question of appropriate control cell lines to determine disease-specific iPS cell phenotypes with new technologies. For example, genetic rescue of iPS cell lines by either expressing wildtype genes or correcting the endogenous genomic sequence.

In summary, human iPS cells can be used to determine disease vulnerability (or resistance) based on cell types. The objective of this approach is to translate this new knowledge of PD related human cellular phenotypes contributed by genetic risk load, pathogenic stressors that precipitate PD, and the phenotypic landscape of cell loss and degeneration that characterizes PD into therapeutic discovery.

Acknowledgements

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