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Reviewer's code: 03739868

SPECIFIC COMMENTS TO AUTHORS

This is an engaging paper presenting an overview of the potential of human iPSCs to provide a valuable tool for mechanistic study and drug discovery in PD research. The presentation of results is somewhat confusing, as outlined below, and should be addressed before publication of this article.

1. The sentence "The injection of 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or the pesticide rotenone into the brain of rats and mice is commonly used in neurotoxin-based in vivo models[30-32]." is only correct with regard to the 6-OHDA-induced PD model. In the other two models, animals are usually injected subcutaneously with rotenone (please refer to reference 32) or intraperitoneally with MPTP (please refer to studies cited in reference 31).

Response:

Thank you very much for your careful review and valuable comment. In the revision, we have revised the sentence as follows "Toxins such as 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), paraquat and rotenone are conventionally used in PD modeling[30]. Commonly, 6-OHDA is stereotactically injected into animal brains and the other toxins are injected subcutaneously or intraperitoneally to induce PD models[31-33] ". One more reference has been added to address toxins-induced PD models.

2. Please improve the sentence "Moreover, there are rat strain differences in response to rotenone, suggesting that rotenone may induce atypical parkinsonism from nonselective neuronal death" since the differences in animals' responses to treatments exist not only between different strains but also between labs as well as being due to

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many factors, such as different protocols of treatment, including doses, frequency, duration, etc., different ages and weights of animals and many others. Of note, the mentioned rat strain differences in response to rotenone are not described precisely or supported by any reference.

Response:

Thank you very much for your comments. We fully agree that the mentioned rat strain differences in response to rotenone are not described precisely and we cannot find any convincing reference to support this claim. In the revision, we have modified the sentence as follows "These neurotoxins can be taken up by DA neurons via dopamine transporters and cause neuronal damage[34]. Their toxicities are possibly due to the inhibition of complex I of the mitochondrial electron transport chain, which leads to depletion of ATP and an increase in reactive oxygen species (ROS), and eventually results in neuronal death[35]. Although these toxins can destroy DA neurons in the SNpc, 6-OHDA and MPTP treatment does not yield aggregation of α-synuclein (Lewy bodies), which is a major pathological marker of PD[36]. Typical PD is a type of chronic neurodegeneration. However, 6-OHDA or MPTP causes acute damage, which is not an appropriate model to mimic the pathogenic factors of PD. Chronic exposure to rotenone in rats leads to aggregation of α-synuclein, DA neurodegeneration and behavior defects. A major concern about rotenone-induced models is that, in addition to degeneration of nigral DA neurons, rotenone causes pronounced degeneration in basal ganglia and brainstem nuclei[37], and leads to high systemic toxicity[38] ".

3. In Table 1 the in vitro phenotype "Cellular and secreted α-synuclein protein" provided, based on reference 39, should be clarified regarding changes vs. the normal phenotype (increased?)

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Since there are some modifications on the reference list (one more reference has been added to address toxins-induced PD models), the reference 39 in the original Table 1 is changed to the reference 40 in the revised Table 1. Yes, the cellular and secreted α-synuclein protein is increased regarding the in vitro phenotype. We have clarified this in vitro phenotype as "Increased cellular and secreted α-synuclein protein" in the revised Table 1.

4. In Table 1 the in vitro phenotype "Increased expression of oxidative stress genes" provided based on reference 52 should be rephrased (oxidative stress-related genes?) In addition in the context of the potential clinical application of patient-derived iPSC-based models of PD, it would be valuable to provide information about

4.1. sources of the iPSCs and clinical phenotypes in Table 1

4.2. the use of iPSC-derived glial cells

4.3. the use of iPSC in the approach of personalized medicine

Response:

Thank you very much for your nice suggestion. The in vitro phenotype "Increased expression of oxidative stress genes" on reference 53 (initially reference 52) has been rephrased as "oxidative stress-related genes" in the revised Table 1.

4.1 Patients-iPSCs are derived from fibroblasts in most studies listed in Table 1 but the clinical phenotypes are not described in these studies. Therefore, we do not provide the information about the sources of the iPSCs and clinical phenotypes in Table 1.

4.2 Thank you very much for your valuable suggestions on providing the information about the use of iPSC-derived glial cells in the review. In the revision, we described in details iPSCs-derived astrocytes in PD modeling with an additional Table 2 as follows: "Astrocytes are the major group of cells in the central nervous system, with a range of functions that provide both structural and metabolic support for neurons. Accumulating

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evidence suggests that astrocyte dysfunction leads to the pathogenesis of PD, especially familial PD[85-88]. As summarized by Booth et al, mutations in DJ-1, SNCA, PLA2G6, LRRK2 and GBA lead to abnormal glutamate uptake, mitochondrial dysfunction, inflammatory response, water transport defect, and autophagy impairment [85]. Gunhanlar et al[86] found that in a coculture system of astrocytes and neurons at a consistent ratio (60: 40), neuron maturation was distinctly upregulated according to electrophysiological maturity. Accordingly, astrocytes are widely used in implementing cellular modeling approaches to the study of neurodegenerative disorders[87]. Similarly, astrocytes could help DA neurons defend against the neurotoxins and attenuate the mitochondrial dysfunction, as observed by Feng Du et al[88]. Remarkably, the astrocytes and neuron co-culture system improved the outgrowth of neuron markers, and stabilized the mitochondrial function through downregulation of ROS and increased mitochondrial function[88]. On the contrary, astrocytes were also used in inducing PD degeneration phenotypes by Santos et al[89] and di Domenico et al[90]. In Santos et al's research, astrocytes were activated and became inflammatory, and were co-cultured with DA neurons[89], while Domenico et al co-cultured PD-patient-derived astrocytes with normal DA neurons[90]. Normal DA neurons were induced to display apoptosis and multiple system dysfunction after co-culture with dysfunctional astrocytes[89,90]. These compelling findings emphasized that astrocytes may substantially participate in PD pathogenesis (Table 2).

4.3. the use of iPSC in the approach of personalized medicine

It is really a valuable comment that we should describe the use of iPSC in the approach of personalized medicine. In the revision, we address this issue from 3 perspectives: 1) iPSCs-based mechanistic studies for exploring clinical therapeutic strategies; 2) iPSCs as a powerful and convincing drug discovery tool in PD; 3) iPSC-based research enhanced development of cell therapy. For details, please go to the section "POTENTIAL

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APPLICATION OF iPSCs IN PD" in the revised manuscript.

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SPECIFIC COMMENTS TO AUTHORS

In this review, the authors summarized iPSC-based PD models from patient-specific as well as genome-editing-based iPSCs. They contend that these models may provide extensive insights into pathogenic mechanisms of PD. They acknowledged that despite great advances in gene editing, high off-target risk and low efficacy still make it difficult and time-consuming to generate genome-editing-based iPSCs. Thus, improving the efficiency and precision of gene editing is important for generating more isogenic PD-specific iPSCs and control cell lines. Inducing an aged state by long-term culture, overexpression of an aging protein, or small molecules is considered in most iPSC-based age-related PD models. They also acknowledged that iPSCs cannot mimic motor symptoms and some nonmotor symptoms such as depression, agrypnia, hyposmia and impairment of cognition.

1. The latter statement is a critical point, since PD is a failure of an extensive neural and vascular network connections and interactions, and it is not sure how the change(s) in one cell phenotype will reflect a complex disease such as PD.

Response:

Thank you very much for your valuable comment. Yes, it is true that PD is a neurodegenerative disease involving complex pathogenic mechanisms. It is well-documented that PD is an age-related neurodegenerative disease caused by the progressive loss of dopaminergic (DA) neurons in the substantia nigra. Human induced pluripotent stem cells (iPSCs) could provide a promising model to gain mechanistic insights into PD pathogenesis and identify new therapeutic targets. In this review, we mainly discuss about the molecular changes in iPSCs-based PD models either derived from PD patients through reprogramming technology or established by gene-editing

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technology, and the promising application of iPSC-based PD models for mechanistic studies and drug testing. Yes, it is really hard to reflect a complex disease such as PD by studying the changes of iPSCs-derived DA neurons. How to reliably establish iPSC-based models for late-onset PD remains to be resolved; and how to bridge the gap between animal and cell studies should be addressed in the future.

2. While the authors extensively reviewed the literature, they did not make a convincing argument how these in vitro models will lead to in vivo translation to the mechanisms and pharmacological interventions.

Response:

It is really a very important issue that needs to be addressed on how these iPSCs-based PD models will lead to in vivo translation to the mechanisms and pharmacological interventions. Thank you very much for your valuable comment. In the revision, we address this issue from 3 perspectives: 1) iPSCs-based mechanistic studies for exploring clinical therapeutic strategies; 2) iPSCs as a powerful and convincing drug discovery tool in PD; 3) iPSC-based research enhanced development of cell therapy. The details are as follows:

POTENTIAL APPLICATION OF iPSCs IN PD

iPSCs-based mechanistic studies for exploring clinical therapeutic strategies

Due to the ability of human iPSCs to differentiate into human DA neurons and astrocytes, human iPSCs are a promising model for studying the pathogenesis of PD. Compared with neurotoxin-induced injuries, human iPSC-derived DA neurons from sporadic or familiar PD patients, or gene editing could give help us to understand the progressive changes of PD neuronal phenotypes as culture time increases[109,110]. Through this PD iPSCs model, we can verify the possible mechanisms of pathogenesis suggested in previous studies in other cell or animal models. Most importantly, studying

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these PD-iPSC-derived DA neurons could explain how the clinical degenerative features of human DA neurons occur[111,112]. At least the changes in some human familial-PD-iPSC-derived neurons can represent the middle or final stage of PD because this iPSC-derived DA neuronal death occurs with α-synuclein accumulation, which is consistent with the observation in PD patients. Compared with familial PD, the etiology of sporadic PD is still a major challenge because of the multifactorial etiopathogenesis of sporadic PD. Since sporadic PD is complicated, the changes in neuronal phenotype cannot reflect the pathogenic alteration in the whole brain or other systems of sporadic PD patients[113,114].

iPSCs as a powerful and convincing drug discovery tool in PD

Current PD therapies help patients relieve motor symptoms, but do not effectively prevent, slow or halt the progression of PD, particularly in the loss of DA neurons[115]. Neurotoxin-based neurons or animal models are commonly used for anti-PD drug screening. Many drugs based on these artificial models have been developed but do not significantly prevent PD progression[116]. One reasonable explanation is that these neurotoxins that usually cause strong injuries in DA neurons cannot mimic the progressive death of human DA neurons in PD. In addition, DA neurons from animals are distinct from human DA neurons. In PD-iPSC-derived DA neurons, the typical PD features such as accumulation of α-synuclein, progressive degeneration, and death could be observed. These robust and reproducible PD phenotypes are amenable to screening potential compounds. Thus, PD-iPSC-derived DA neurons are more suitable for screening anti-PD drugs than artificial models are.

iPSC-based research enhanced development of cell therapy

The inspiring success in PD treatment was achieved through allograft of human fetal midbrain cell suspensions in 1980[117]. In addition, in MPTP-treated monkey brains, monkey ESC-derived neural progenitor cells differentiated into DA neurons and cells

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integrated well in the striatum, thereby PD-related motor symptoms improved[118]. Compared with ESCs, iPSCs have more potential in cell replacement therapies for PD because they can be generated from patients' own cells and differentiate into DA neural progenitor cells that specifically develop into DA neurons[119]. iPSCs have the advantage of eliminating immune rejection concerns as they are obtained from the host. The generation of iPSCs from a patient's own somatic cells would potentially allow for a plentiful source of cells for autotransplantation. In addition, using iPSCs rather than ESCs means that this treatment would be potentially available in some countries that ban the application of ESCs, including Italy, Ireland, and most African and South American countries[120,121]. For familial PD patients, the corrected iPSCs are also a reasonable source for the transplantation of normal DA neurons to reduce motor symptoms. Recently, scientists from Japan have started a clinical trial[122] (ClinicalTrials.gov NCT02452723) to treat PD with human iPSCs. All these suggest that the transplant of human iPSCs-derived DA neurons will be a promising therapeutic strategy and customized treatment is practical due to individual differences.