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RESEARCH HIGHLIGHT

# Huntington's disease: Dancing in a dish

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**In a recent landmark paper, the Huntington's disease (HD) iPSC Consortium reports on the establishment and characterization of a panel of iPSC lines from HD patients, and more importantly, the successful modeling of HD *in vitro*. In the same issue of *Cell Stem Cell*, An *et al*. reports on the successful targeted gene correction of HD in human iPSCs. Both advances are exciting, provide new resources for current and future HD research, and uncover new challenges to better understand and, most importantly, treat this devastating disease in the near future.**

Modeling human diseases using induced pluripotent stem cells (iPSCs) has created novel opportunities for both mechanistic studies as well as for the discovery of new disease therapies. Combined with advanced gene correction technology, human iPSCs hold great promise to provide patient-specific and mutation-free cells for potential cell replacement therapy. Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder, which causes motor dysfunction, psychiatric disturbances and cognitive impairment [1]. HD is caused by an expanded cystosine adenine guanine (CAG) tri-nucleotide repeat encoding polyglutamine in the first exon of the Huntingtin (*HTT*) gene. To date, there is no effective therapy for preventing the onset or slowdown of this disorder. Preliminary clinical trials using fetal neural grafts had shown long-lasting functional benefits in patients [2]. Though only effective in limited cases, these results suggest that cell-based therapy could be a potential treatment if a reliable and consistent cell source is available. For this purpose, an alternative cell source to overcome the logistical and biological hurdles of this disease had been actively explored in the past decade. With recent advancement in human iPSCs technology, HD patient-specific iPSCs coupled with an efficient directed cell differentiation protocol offers hope for an unlimited supply of autologous cells. Since HD is a monogenic disease, with a very well-established correlation between the number of CAG repeats and the age of disease onset, it provides an ideal target for iPSC-based gene correction that will allow for the production of disease-free cells for potential autologous cell therapy, and at the same time provide a much needed, valuable platform to further study the pathogenesis of the disease [3, 4].

This is in fact what has been recently accomplished in two reports published in *Cell Stem Cell* [5, 6]. The HD iPSC Consortium reports on the generation of HD patient-specific iPSC lines that showed CAG-repeat-expansion-associated phenotypes [5]. The study from An *et al*. [6] reports on the successful targeted correction of expanded CAG repeat in HD patient iPSCs and the reversion of disease phenotypes.

In the study reported from the HD iPSC Consortium, the authors generated 14 iPSC lines from HD patients and controls (listed in Table 1). These HD iPSCs group into three types: control, medium CAG repeat, and long CAG repeat iPSC lines. When differentiated into neural lineages, both medium and long CAG repeat HD lines showed similar pathological profiles in electrophysiology, energy metabolism, cell adhesion and cell death, which were significantly different from the control group. However, only long repeat lines exhibited robust pathologic phenotypes in response to cellular stress and BDNF withdrawal. More interestingly, the authors developed an assay based on calcium homeostasis following repeated glutamate pulsing, which showed CAG-repeat-expansion-dependent phenotypes across different iPSC lines. In addition, the HD iPSC Consortium provided gene expression profiles of neural stem cells (NSCs) and striatal-like cells differentiated from different HD iPSCs or controls, and thus a good resource for future searching for candidate HD contributing factors. The study reported by the

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**Table 1** Existing iPSC lines derived from patients with Huntington's disease

| Code | Number of iPSC line | CAG repeats | HD type | Age of sample procured | Reprogramming strategy | Phenotype detected cell type | Gene correction line available | Phenotype | References |
|---|---|---|---|---|---|---|---|---|---|
| HD 43 | 1 | 39/43 | Adult onset HD | 44 years | OSKM (lentivirus) | iPSCs | no | Increased lysosomal activity | [7] |
| HD 44 | 4 | 42/44 | Adult onset HD | 59 years | 2 lines:OSKM (lentivirus)<br>2 lines: OSK (lentivirus) | iPSCs | no | Increased lysosomal activity | [7] |
| HD 50 | 1 | 50 | Adult onset HD | unknown (father) | OSKM (retrovirus) | Astrocyte | no | Neural differentiation normal, Vacuolation in astrocyte | [12] |
| HD109-1 | 1 | 109 | Juvenile HD | unknown (daughter) | OSKM (retrovirus) | Astrocyte | no | Similar to HD 50, more vacuolation in astrocyte | [12] |
| HD 72 | 1 | 72 | Juvenile HD | 20 years | OSKM (retrovirus) | NPCs | yes | Elevated caspase activity; more vulnerable to cell death | [6, 8, 9] |
| HD 60 | 3 | 60 | Adult onset HD | 29 years | 2 lines:OSKMNL (lentivirus)<br>1 line: OSKM (episomal) | NPCs, neurons | no | Altered cell adhesion, energetics, and electrophysiology; Increased cell death in long time neural differentiation | [5] |
| HD109-2 | 1 | 109 | Juvenile HD | 9 years | OSKMNL (lentivirus) | NPCs, neurons | no | Similar to HD 60; higher risk to cell death in response to BDNF withdrawal | [5] |
| HD180 | 4 | 180 | Juvenile HD | 6 years | 3 lines:OSKMNL (lentivirus)<br>1 line: OSKM (episomal) | NPCs, neurons | no | Similar to HD 60 and 109; Increased vulnerable to stress and toxicity | [5] |

HD, Huntington's Disease; iPSC, induced pluripotent stem cell; NPC, neural progenitor cell; O, Oct4; S, Sox2; K, Klf4; M, cMyc; N, Nanog; L, Lin28.

HD iPSC Consortium was conducted by eight individual research groups. The diversity of patient backgrounds, sample origins and CAG-repeat lengths of iPSCs lines certainly helped to reduce the individual bias in the study. The consistency in the observations reported by the different research groups further strengthens the reliability of the results. With these exciting phenotypes and remarkable results presented, the HD iPSC Consortium offers the field an HD iPSC collection representing a unique and well-characterized model for 'recapitulating HD in a dish'. Moreover, the model provides a comprehensive human cell platform for the future screening of new candidate therapeutics against HD.

Meanwhile, using a homologous recombination-based gene targeting strategy, An *et al*. [6] reported on the successful correction of the CAG-repeat-expanded *HTT* allele in HD patient iPSCs. These corrected iPSCs shared the same genetic background as the disease iPSCs, thereby serving as non-biased controls for their uncorrected counterparts. By comparing gene expression profiles of corrected iPSCs versus disease iPSCs, An *et al*. found that the alterations of cadherin, TGF-β, and caspase-related pathways in HD were rescued in the non-expanded iPSCs. The authors further demonstrated that gene correction in HD iPSCs reversed disease phenotypes such as susceptibility to cell death and altered mitochondrial bioenergetics in NSCs. More importantly, when transplanted into a mouse model of HD, the corrected HD iPSC-derived NSCs could survive and differentiate into GABAergic neurons and DARPP-32-positive neurons *in vivo*.

Taken together, these two studies present very significant advances for iPSC-based disease modeling of HD and provide a potential donor source for cell replacement therapy. Though exciting indeed, several important challenges remain unsolved.

First, complete recapitulation of

neuropathology phenotypes in the iPSC-based models *in vitro* remains a challenge in the field. As a neurodegenerative disease, pathologic development of HD usually takes several decades and may be influenced by several external factors. In the HD iPSC-based model, the derivation method, clonal discrepancy as well as the culture conditions may affect the manifestation of phenotypes. Indeed, in previously reported HD iPSC lines, only slight increases in caspase and lysosomal activity were observed [7-9]. Although in both reports of HD iPSCs, significant phenotypes in electrophysiology, energy metabolism and cell death were recorded, other typical HD-associated phenotypes such as oligomeric mutant HTT aggregation, formation of nuclear inclusions and preferential striatal degeneration were not observed.

Second, it is still an open question whether neural cells derived from gene-corrected iPSCs are fully functional, that is, whether they may restore physiological functions after cell replacement therapy. Ma *et al*. [10] have recently reported on a protocol to differentiate striatal projection neurons from human embryonic stem cells with a high efficiency. After transplantation, these cells survived, reconnected striatal circuitry, and restored motor function in a striatal neurodegenerative mouse model. In spite of these encouraging first attempts, further improvements of the methodology for the directed cell differentiation *in vitro* and cell transplantation *in vivo* are still needed.

Third, HTT protein is ubiquitously expressed and functional in different tissue. It has been hypothesized that HD may also develop in a non-autonomous manner [11]. The current studies mainly focused on the phenotypes of HD iPSC-derived neurons. However, supporting cells such as astrocytes might also play direct or indirect roles in HD progression. Indeed, a vacuolation phenotype has been observed in HD iPSC-derived astrocytes [12]. Therefore, it will be interesting to expand the HD iPSC platform into other cell types with the goal to extend and uncover the various ethiopathological factors involved in HD.

Finally, human iPSC models of monogenic disorders in general possess great potential for the mechanistic study of the disease. However, as is the case with many neuropsychiatric disorders, HD establishment and progression is linked to different genetic and epigenetic factors, including environmental change-induced epigenetic modification, multiple mutations, and genetic alternation in non-coding regions. To this end, although the successful generation of HD iPSCs as well as targeted gene correction would greatly facilitate the study of HD, a comprehensive understanding of HD pathogenesis will need to be achieved before trying to translate the recent results into the clinic.

In summary, despite all of these open questions, the recent studies have uncovered the unlimited potential of iPSCs for modeling HD *in vitro*. These studies will promote and enhance HD research in various areas, including elucidation of HD cellular pathogenesis, development of HD-specific biomarkers, screening for small therapeutic molecules, and manipulation of HD iPSCs for stem cell replacement therapy, which together may ultimately fulfill the promise of using iPSCs as a tool for regenerative medicine and drug discovery for HD in the near future.

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