PERSPECTIVE

Induced pluripotent stem cells from Huntington's disease patients: a promising approach to define and correct disease-related alterations

Adult somatic cells such as skin or blood cells from either health donors or patients can be reprogrammed into induced pluripotent stem cells (iPSCs). Given their unlimited self-renewal and differentiation capacities, iPSCs are an invaluable resource to generate terminally differentiated cells. Thus, iPSCs can facilitate the study of human diseases and drug screening, holding great promise for regenerative medicine. Another significant advantage of iPSC disease-modeling is that normal and mutant proteins are expressed at endogenous levels. In addition, subtle phenotypes and the effects of genetic background variations can be assessed by comparison between iPSC lines obtained from different patients and healthy donors as well as isogenic lines, in which disease-related mutations are corrected.

As with other multiple diseases, iPSCs derived from Huntington's disease (HD) patients provide an opportunity to define disease-related changes and possible interventions to correct these alterations. HD is an autosomal dominant neurodegenerative disorder characterized by cognitive deficits, psychosis and motor dysfunction (Finkbeiner, 2011). HD is caused by mutations that extend the cytosine-adenine-guanine (CAG) trinucleotide repeat in the exon 1 of the *huntingtin* (*HTT*) gene. 34 CAG repeats or fewer do not result in HD symptoms. Alleles containing 35–39 CAG repeats produce incomplete penetrance, as individuals harboring these alleles may or may not develop the disease. However, > 39 CAG repeats is considered fully penetrant as individuals with these alleles will eventually develop HD symptoms. In this regard, the length of the CAG repeats correlates with the disease progression and longer CAG stretches predict younger HD onset (Finkbeiner, 2011).

Expanded CAG mutations result in an unstable polyglutamine (polyQ) stretch in HTT protein, leading to its aberrant aggregation. As such, the accumulation of mutant HTT aggregates is one of the hallmarks of the disease. Although HTT is ubiquitously expressed, gamma-aminobutyric acid (GABA)ergic medium spiny neurons of the striatum undergo the greatest neurodegeneration in HD patients (Finkbeiner, 2011). Extensive data indicate that mutant HTT aggregation is toxic and contributes to neurodegeneration (Koyuncu et al., 2017). However, the molecular mechanisms by which these inclusions induce neuronal dysfunction remain unsolved. For instance, polyQ-expanded aggregates may collapse distinct proteostasis nodes such as protein clearance mechanisms (*i.e.*, autophagy or the ubiquitin proteasome system) or the chaperone network (Koyuncu et al., 2017). Moreover, aberrant aggregates could sequester signalling and regulatory components such as transcription factors or physically obstruct neuronal extensions. Besides aggregates, growing evidence indicates that intermediate species called "oligomers" formed during the aggregation or disaggregation process also contribute to neurotoxicity. In this regard, the initial formation of polyQ-expanded HTT inclusions has been proposed to have a protective role. These aggregates could form to sequester highly toxic oligomers of mutant HTT, reducing the amount of soluble oligomeric intermediates (Arrasate et al., 2004). However, mutant HTT inclusions may eventually sequester other proteins, contributing to neurodegeneration.

iPSCs derived from HD patients express significant amounts of mutant HTT protein (Koyuncu et al., 2018). Whether HD-iPSCs exhibit toxic soluble oligomers of mutant HTT is unknown. However, HD-iPSCs do not exhibit increased cellular death, higher sensitivity to cellular stressors or defects in GABAergic neuronal differentiation (Koyuncu et al., 2018), suggesting that these cells have increased mechanisms to either avoid the generation of toxic oligomers or eliminate them. Nevertheless, extensive evidence indicates that HD-iPSCs suppress the accumulation of mutant HTT aggregates (Koyuncu et al., 2018), which are important determinants of cellular viability and function. These findings suggest a rejuvenation process during cell reprogramming that rewires the ability to maintain proteostasis of mutant HTT, resulting in iPSCs with increased mechanisms to prevent aberrant aggregation. For this reason, HD-iPSCs have been used to define anti-aggregation mechanisms, which can be then mimicked in differentiated neurons to suppress polyQ-expanded HTT aggregation (Koyuncu et al., 2018). For instance, this research led to identify novel activators of mutant HTT degradation as well as inhibitors of aggregation (Koyuncu et al., 2017). On the other hand, the rejuvenation step also represents an important limitation for HD disease modeling. Although HD-iPSCs can terminally differentiate into striatal neurons, these cells do not exhibit mutant HTT aggregates (Koyuncu et al., 2018). The lack of polyQ-expanded HTT aggregates in these cells could reflect the long period of time before aggregates accumulate in the neurons of HD patients. In support of this hypothesis, HD-neurons derived from iPSCs do not present polyQ-expanded aggregates at 12 weeks after transplantation into HD rat models, whereas they accumulate aggregates after 33 weeks. Notably, recent advances have provided a novel tool to circumvent this limitation and bypass the induction of pluripotency. In particular, this approach allows for direct conversion of fibroblasts from HD patients into neurons that recapitulate age-associated aggregation of mutant HTT (Victor et al., 2018).

Besides aggregation of mutant HTT, loss of normal HTT function could also contribute to HD (Saudou and Humbert, 2016). In these lines, downregulation of wild-type HTT levels induces HD-related changes such as progressive neurodegeneration and motor dysfunction or aggravate these changes in HD models. Moreover, overexpression of wild-type HTT improves brain cell survival and ameliorates the deleterious effects of the mutant protein (Saudou and Humbert, 2016). Although differentiated cells from HD-iPSC do not exhibit aggregates, they have other disease-related changes such as alterations in electrophysiology and calcium homeostasis, increased vulnerability to excitotoxic stressors as well as cumulative risk of death over time (HD iPSC Consortium, 2017). Notably, neurons differentiated from HD-iPSCs also present altered morphology and maturation phenotypes (Mehta et al., 2018). These findings support a direct link of HTT with regulation of development. Indeed, downregulation of HTT at early developmental stages results in neural differentiation defects. Remarkably, cumulative evidence indicates developmental deficits in HD mouse models and human pathological specimens. For instance, mutant HTT expression correlates with an impairment in the maintenance of striatal neural stem cells and their specification into medium spiny neurons of the striatum in knock-in (polyQ111) mice (Molero et al., 2009). In addition, HD patients as well as YAC128 mice expressing human polyQ128-HTT exhibit alterations in brain morphology and synaptic plasticity before the onset of disease symptoms (Milnerwood and Raymond, 2010).

Interestingly, both neural and neuronal cells derived from HD-iPSCs exhibit pronounced HD-related transcriptional changes consistent with those reported in the brain of HD patients, such a dysregulation of neurodevelopmental transcription factors and genes involved in axonal guidance, calcium influx, voltage-gated sodium currents or GABA signaling (HD iPSC Consortium, 2017; Mehta et al., 2018). In fact, transcriptional dysregulation is a major defect observed in postmortem HD brains and HD mouse models (Saudou and Humbert, 2016). Gene expression dysregulation can ensue from epigenetic alterations. In these lines, several studies indicate HD-related modifications in epigenetic marks such as DNA methylation and post-translational modifications of histone proteins, which can directly contribute to aberrant gene expression in HD patients as well as cellular and organismal models (Saudou and Humbert, 2016). Among these epigenetic changes, H3K9 trimethylation (H3K9me3) is robustly increased in HD patients and transgenic R6/2 HD mice (Ryu et al., 2006). H3K9me3 is linked with heterochromatin formation and correlates with transcriptional repression. Remarkably, neural cultures differentiated from HD-iPSCs have epigenetic alterations (HD iPSC Consortium, 2017), including an increase in H3K9me3 levels (Irmak et al., 2018).

Given the strong link of HTT with neural development and the control of the epigenetics landscape, the study of pluripotent stem cells can shed light on the mechanisms underlying this process. Because epigenetic marks are reversible, research on HD-iPSCs can also lead to novel interventions to correct epigenetic and differentiation alterations at earlier developmental stages, which can contribute to disease phenotypes. For this purpose, we first focused on under-

standing the normal function of HTT in human pluripotent stem cells. In these cells, the levels of gene expression-repressive marks such as H3K9me3 are usually reduced while they maintain their pluripotent state. By studying the normal function of HTT, we identified the chromatin factor ATF7IP as an interactor of HTT protein (Irmak et al., 2018). ATF7IP interacts and regulates SETDB1 methyltransferase, facilitating the trimethylation of H3K9. Notably, we find HTT inhibits the interaction of the ATF7IP-SETDB1 complex with other key regulators of heterochromatin and transcriptional repression, maintaining low levels of H3K9me3 in pluripotent stem cells. When the levels of HTT are reduced, the ATF7IP-SETDB1 complex gains interactions with transcriptional repressors (DNMT3B, SLTM, GATAD2A/GATAD2B) and heterochromatin regulators (SMARCC2, PBRM1, SMARCAD1, HP1B3, HMGA1, WDR82). Concomitantly, loss of HTT results in a dramatic increase in H3K9me3 levels in control pluripotent stem cells. Knockdown of HTT not only promotes global increase of H3K9me3 levels but also enrichment of H3K9me3 marks at distinct genes, including transcriptional regulators of neuronal differentiation such as ASCL2 and GBX1. Although these H3K9me3 repressive marks do not have strong effects at the pluripotent state, they have detrimental effects at later differentiation stages. Since abnormal levels of H3K9me3 are conserved during differentiation into progenitor cells, they repress the induction of neurogenic factors required for terminal differentiation and maturation into neurons. Thus, loss of HTT can induce alterations in H3K9me3 repressive marks at the pluripotent state, eventually reducing the induction of neural and neuronal genes during differentiation. These findings indicate that interventions for correcting epigenetic changes should be applied at the pluripotent state to avoid differentiation into cells with a compromised transcriptome.

Since our results indicated a role of HTT in the inhibition of the ATF7IP-SETDB1 complex, we asked whether polyQ-expanded mutations alter this normal function of HTT (**Figure 1**). Strikingly, expanded polyQ repeats block the interaction of HTT with the ATF7IP-SETDB1 complex, leading to its activation and concomitant increase of H3K9me3 levels. When HD-iPSCs are differentiated into neural cells, these cells exhibit dramatic alterations in the expression of multiple genes involved in neuronal differentiation and maturation. Prompted by these findings, we asked whether decreasing ATF7IP levels could diminish trimethylation of H3K9 in neural cells derived from HD-iPSCs. Indeed, we found that knockdown of ATF7IP after differentiation is sufficient to reduce H3K9me3 levels in HD neural cells. Since HD-iPSCs differentiate into cells that already present HD-related changes, one step further was to reduce H3K9me3 levels at the iPSC level prior inducing differentiation. Importantly, loss of ATF7IP at the iPSC stage allows for the generation of neural cells with rescued H3K9me3 levels and expression of genes involved in neuronal function. Thus, modulation of ATF7IP can ameliorate alterations in the expression of multiple genes, particularly those which are normally diminished in HD.

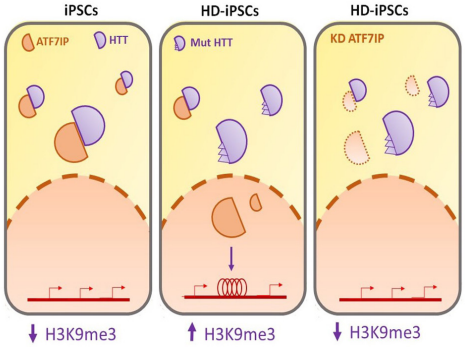

Figure 1 Mechanism suppressing H3K9 trimethylation in pluripotent stem cells and its demise by expanded-polyQ HTT mutations.
In normal conditions, HTT interacts with ATF7IP to reduce H3K9 trimethylation of iPSCs. In HD-iPSCs, mutant expanded polyQ repeats in HTT protein diminish its interaction with ATF7IP, a process that results in up-regulation of H3K9me3 marks and transcriptional repression of genes required for neuronal differentiation and maturation. Loss of ATF7IP rescues alterations in H3K9me3 and gene expression. HD: Huntington's disease; HTT: huntingtin; iPSCs: induced pluripotent stem cells; polyQ: polyglutamine.

Altogether, by using iPSCs we identified ATF7IP as a potential target to correct aberrant epigenetic marks and gene expression induced by mutant HTT. Likewise, the HD-iPSC consortium also identified that a small molecule, isoxazole-9, targets several of the dysregulated gene networks in neural cells from HD-iPSCs (HD iPSC Consortium, 2017). Remarkably, isoxazole-9 treatment normalizes gene expression in HD-iPSC-derived neural cells. In addition, isoxazole-9 can rescue cognition and synaptic pathology in R6/2 HD mouse model (HD iPSC Consortium, 2017). Besides their application for the understanding and correction of dysregulated gene networks, we have also demonstrated that HD-iPSCs can be an important resource to define regulators of proteostasis of mutant HTT, suppressing its aggregation and toxicity. These promising findings further support the use of HD-iPSCs to understand HD. It will be fascinating to follow the advances in the study of HD-iPSCs and their differentiated counterparts, which can lead to novel interventions for HD.

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Azra Fatima, Ricardo Gutiérrez-Garcia, David Vilchez*
Institute for Genetics and Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany
***Correspondence to:** David Vilchez, PhD, dvilchez@uni-koeln.de.*
***orcid:** 0000-0002-0801-0743 (David Vilchez)*
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