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Modeling Huntington's disease with induced pluripotent stem cells

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ABSTRACT

Huntington's disease (HD) causes severe motor dysfunction, behavioral abnormalities, cognitive impairment and death. Investigations into its molecular pathology have primarily relied on murine tissues; however, the recent discovery of induced pluripotent stem cells (iPSCs) has opened new possibilities to model neurodegenerative disease using cells derived directly from patients, and therefore may provide a human-cell-based platform for unique insights into the pathogenesis of HD. Here, we will examine the practical implementation of iPSCs to study HD, such as approaches to differentiate embryonic stem cells (ESCs) or iPSCs into medium spiny neurons, the cell type most susceptible in HD. We will explore the HD-related phenotypes identified in iPSCs and ESCs and review how brain development and neurogenesis may actually be altered early, before the onset of HD symptoms, which could inform the search for drugs that delay disease onset. Finally, we will speculate on the exciting possibility that ESCs or iPSCs might be used as therapeutics to restore or replace dying neurons in HD brains.

Contents

Introduction

Huntington's disease (HD) is a devastating and incurable neurological disorder caused by a CAG expansion in the gene encoding the protein huntingtin (Htt). HD is inherited in an autosomal-dominant

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fashion and leads to sleep disturbances (Arnulf et al., 2008), motor dysfunction, cognitive impairment and psychiatric abnormalities (Vonsattel and DiFiglia, 1998) and ultimately premature death (DiFiglia et al., 1995). The age of onset (typically 30–50 years) and severity correlate with the number of CAG-encoded glutamine repeats in mutant Htt (mHtt) (Andrew et al., 1993; Snell et al., 1993), whereby normal, unaffected individuals have 35 or fewer CAG repeats while affected individuals have more than 36 (Langbehn et al., 2004). Individuals with longer expansions have an earlier onset of disease (Langbehn et al., 2004). Cellular pathologies associated with HD result in the death of striatal medium spiny neurons (MSNs) in the basal ganglia (Augood et al., 1997), the executive center for orchestrating motor memory, learning and function in the brain (Packard and Knowlton, 2002). At later stages mHtt also affects other cell types, such as cortical neurons and pyramidal projection neurons (Han et al., 2010). Although the mutation that causes HD is known, no therapies that delay or slow the disease progression currently exist (Mestre et al., 2009).

Htt participates in numerous biological processes, such as regulating apoptosis (Hickey and Chesselet, 2003), regulating transcription and enhancing microtubule-based transport or scaffolding of cytoskeletal molecules at synapses (Cattaneo et al., 2005). Hallmarks of mHtt-affected cells include abnormal gene expression (Cha, 2007; Thomas, 2006), aberrant protein folding, degradation and clearance (Finkbeiner, 2011), and the formation of large protein aggregates called inclusion bodies (DiFiglia et al., 1997).

It has been hypothesized that the polyglutamine expansion in mHtt alters its conformation, leading to aggregation and abnormal protein-protein interactions (Miller et al., 2010) and may also alter Htt's normal function (Kratter and Finkbeiner, 2010). In turn, misfolded mHtt may disrupt cellular homeostasis by overwhelming the cell's proteostasis machinery by interfering with protein-clearance pathways (Finkbeiner, 2011). The accumulation of misfolded forms of mHtt may also stress protein homeostasis pathways so that unrelated proteins cannot fold or function properly, thus compromising cell health. As a consequence, the cell sequesters mHtt through the formation of IBs that help mitigate the stress on the proteostasis network by aggregating mHtt into forms that are more inert than misfolded monomers (Arrasate et al., 2004). In addition, mHtt is known to interact promiscuously with other molecules, and thereby may impede their expression or change their function (Li and Li, 2004). For example, mHtt interferes with CREB activation of its gene targets (Sugars et al., 2004). Thus, mHtt disrupts multiple fundamental cellular functions, making it challenging to conceive of simple therapeutic strategies.

As with other neurodegenerative diseases, we have learned a great deal about HD from transgenic animals and rodent primary neuronal cultures. However, as diseased human cells are not easily obtainable, much of the work to understand the molecular insults of mHtt has been done in murine tissues. Despite successful modeling of several aspects of HD *in vitro* and *in vivo*, therapies that appeared beneficial in these models have been disappointing in human clinical trials.

Until recently, studies in human tissue have been restricted to immortalized or transformed cells, fixed post-mortem tissues and blood samples. While valuable in some respects, immortalized cells have fundamentally different biological properties than brain cells and fixed tissues are subject to the degradation of DNA and RNA. Blood samples have been valuable for detecting gene-expression changes that can be used as biomarkers of disease progression (Borovecki et al., 2005; Long et al., 2012; Lovrecic et al., 2009) but these changes only recapitulate some key molecular characteristics. Further, the availability of such tissues is limited. The dearth of robust human tissue-based systems derived directly from HD patients has slowed the validation of promising therapeutics.

The discovery of induced pluripotent stem cell (iPSC) technology provides exciting new opportunities to develop *in vitro* HD models that more accurately reflect the human disease. Cells obtained directly from HD patients can be propagated indefinitely and differentiated into the susceptible neuronal subtypes. Although HD is a late-onset disease, recent evidence suggests that these comparatively young HD iPSCs can accurately recapitulate several aspects of the human disease (An et al., 2012; The HD iPSC, 2012). Here, we will examine the practical implementation of iPSCs to study HD, including methods to differentiate embryonic stem cells (ESCs) or iPSCs into MSNs, the cell type most susceptible to the pathogenic effects of mHtt. We will explore the HD-related phenotypes identified in iPSCs and ESCs, and review how brain development and neurogenesis may be altered early, before the onset of HD symptoms. Finally, we will explore the exciting possibility that ESCs or iPSCs might be used as therapeutics to restore or replace dying neurons in HD brains. The development of human HD models using iPSCs could lead to critical advances in our understanding of HD progression at its earliest stages, and in turn, could lead to the development of relevant small-molecule or cell-based therapeutics that delay disease onset and progression.

Modeling human disease in rodents

While transgenic animals and rodent primary neuronal cultures have been valuable systems for studying HD, therapies developed using these models have been disappointing in human clinical trials. Clearly, rodents are not humans, having diverged ~96 million years ago (Nei et al., 2001), and the cumulative effect of small and infrequent base-pair changes over time might affect how a putative small-molecule therapeutics interacts with that cell's proteome. Thus, an effective drug in rodent cells may not work as well or as specifically in humans. Furthermore, many single amino-acid polymorphisms alter molecular stability and global protein–protein interactions (Ferrer-Costa et al., 2002). For example, two single-nucleotide polymorphisms in *APOE4* increase the risk of Alzheimer's disease and significantly alter APOE structure and protein function (Mahley et al., 2006). Further, these polymorphisms yield different functional consequences of protein toxicity in the human versus the rodent (Vance and Hayashi, 2010).

In addition to functional differences between human and rodent proteins, the complexity of the human brain far exceeds that of the rodent at the cellular and systems levels. Thus, there are many examples in which murine models do not recapitulate the physiology or anatomy of the human brain (Dolmetsch and Geschwind, 2011). For example, anatomically the human brain is simply bigger and far more complex in shape than that of a mouse. There are significant differences in the development and patterning of the cerebral cortex and subventricular zones (Clowry et al., 2010; Dolmetsch and Geschwind, 2011) (Table 1). The human brain contains more glial subtypes and a higher ratio of glia to neurons than rodents (Oberheim et al., 2006) while some human neuronal subtypes appear to be completely lacking in rodents altogether (Table 1).

In terms of gene-expression profiles in specific cell types, humans and mice can have global differences in the expression of genes involved in pluripotency, cell-cycle regulation, and apoptosis (Ginis et al., 2004) as well as in innate and adaptive immunity (Mestas and Hughes, 2004) and response to inflammatory stress (Zermeno et al., 2009). While some reports show a high conservation in expression of orthologous genes (Zheng-Bradley et al., 2010) there are many examples of divergence of gene function, alternate splicing events and difference in copy number variants between mice and humans (Gharib and Robinson-Rechavi, 2011) (Table 1). In addition, binding sites for highly conserved transcription factors can vary significantly between rodents and humans (Odom et al., 2007). Thus, it is not surprising that so many drugs fail once they reach clinical trials given the heavy reliance on model systems that are so different from human tissues.

Does the HD mutation affect brain development?

Historically, HD has been thought of as a late-onset disorder, with the initiation of the disease marked by the first appearance of

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