INTERROGATING NEURODEGENERATIVE DISEASE STATES USING PLURIPOTENT STEM CELLS: A CASE OF STUDY IN HUNTINGTON DISEASE

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ABSTRACT

Evidence is mounting that the propagation of missfolded proteins in neural networks contributes to disease progression in neurodegenerative disorders. Underlying mechanisms remain largely unknown. Here we show for the first time that mutant Huntingtin (mHtt) aggregates propagate in a transneuronal fashion. We demonstrate this by using a novel co-culture system of wild type human embryonic stem cell (hESC)-derived neurons in organotypic mouse brain slices (OTBSs). This environment allows hESC-derived neurons to functionally mature into subtypes matching local network specifications. In OTBSs from R6/2 HD model but not wild type mice, hESC-derived neurons show non cell-autonomous atrophic changes and acquire mHtt aggregates from their R6/2 mouse neighbors using synaptic vesicle machinery. In mixed-genotype OTBS cultures of R6/2 cortex and wild type striatum we show that mHtt aggregates propagate in the for Huntington's disease (HD) clinically relevant corticostriatal connection. Transsynaptic propagation of mHtt in neural circuits using synaptic machinery likely is a previously underestimated contributor to disease progression and may offer novel opportunities for targeted therapy.

KEYWORDS
Embryonic stem cell, Huntington disease, synaptic transmission, protein aggregate.

INTRODUCTION

Huntington's disease (HD) is an autosomal-dominant neurodegenerative disorder caused by an increased number of CAG repeats in the huntingtin gene (1993). The disease relentlessly progresses until death and no treatment so far has been shown to delay disease onset or progression.

A histopathological hallmark that HD shares with other neurodegenerative disorders including Prion's disease, Alzheimer's disease (AD) and Parkinson's disease (PD) is an abnormal deposition of protein aggregates. The contribution of these aggregates to disease onset and/or progression remains controversial, with views ranging from aggregates impairing normal cellular functions (Lorenzo and Yankner, 1994; Thomas et al., 1996) to having no major role in pathogenicity (Kim et al., 1999; Leavitt et al., 1999) or protecting cells by scavenging toxic soluble mHtt species (Ross, 2002).

Evidence has been mounting in several neurodegenerative diseases that, like in Prion's disease, misfolded protein pathologies spread across brain regions. Examples include Aβ and Tau pathology in AD and α-synuclein pathology in PD (references). mHtt and other poly-glutamine (poly-Q) proteins also seem capable of intercellular transmission, at least in non-neuronal cells, but so far mHtt has not been shown to propagate from neuron to neuron and in neural circuits (Herrera et al., 2011; Ren et al., 2009).

Mechanisms proposed to facilitate the propagation of misfolded protein aggregate pathologies include tunneling nanotubes, exocytosis/endocytosis including the involvement of exosomes or via the neurotransmission machinery (Lee et al., 2010). Endocytosis appears critical for propagating e.g. α-synuclein pathology (Desplats et al., 2009) whereas mHtt aggregates have unique physical properties that seem sufficient to passively cross the membrane, penetrate mammalian cells and gain access to the cytoplasm (Ren et al., 2009).

Increasing evidence also suggests that protein aggregate pathologies spread via functionally connected neural networks and that this process likely contributes to non-cell autonomous damage in neural circuits and disease progression. In HD, cell autonomous and non-cell autonomous pathophysiological changes have been noted, in particular in the corticostriatal pathway. So far, however, there was no evidence that transneuronal propagation of mhtt might contribute to neuronal damage in this circuit that is affected early in HD (for reviews see Raymond et al., 2011).

EXPERIMENT

Here we addressed the fundamental question whether in HD mHtt protein can propagate and contribute to non-cell autonomous pathological changes in neurons. To this end we established a versatile ex-vivo model system of human embryonic stem cell (hESC)-derived neurons co-cultured with mouse organotypic brain slices (OTBSs) from either wild type (wt) or HD model mice (R6/2). We demonstrate that the OTBS supports hESC-derived neurons to functionally mature into subtypes that match local network specifications. Mouse neurons in R6/2 OTBSs progressively accumulate mHtt aggregates and the disease tissue triggers non cell autonomous atrophic changes in co-cultured human neurons that do not occur in co-cultures with wt OTBS. Furthermore, we show that mHtt protein propagates from mouse cells of the R6/2 OTBSs to hESC-derived neurons. This occurs during a specific time window and it requires functional components of the synaptic vesicle shuttling machinery. Moreover, some atrophic changes in hESC-derived neurons correlate with the acquisition of mHtt aggregates. Transneuronal propagation of mHtt can therefore contribute to non-cell autonomous pathophysiological changes in HD and this process might be a so far underestimated contributor and variable in the onset and progression of HD.

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