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Transcriptional profiles for distinct aggregation states of mutant Huntingtin exon 1 protein unmask new Huntington's disease pathways

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ABSTRACT

Huntington's disease is caused by polyglutamine (polyQ)-expansion mutations in the CAG tandem repeat of the *Huntingtin* gene. The central feature of Huntington's disease pathology is the aggregation of mutant Huntingtin (Htt) protein into micrometer-sized inclusion bodies. Soluble mutant Htt states are most proteotoxic and trigger an enhanced risk of death whereas inclusions confer different changes to cellular health, and may even provide adaptive responses to stress. Yet the molecular mechanisms underpinning these changes remain unclear. Using the flow cytometry method of pulse-shape analysis (PulSA) to sort neuroblastoma (Neuro2a) cells enriched with mutant or wild-type Htt into different aggregation states, we clarified which transcriptional signatures were specifically attributable to cells before versus after inclusion assembly. Dampened CREB signalling was the most striking change overall and invoked specifically by soluble mutant Httex1 states. Toxicity could be rescued by stimulation of CREB signalling. Other biological processes mapped to different changes before and after aggregation included NF-kB signalling, autophagy, SUMOylation, transcription regulation by histone deacetylases and BRD4, NAD + biosynthesis, ribosome biogenesis and altered HIF-1 signalling. These findings open the path for therapeutic strategies targeting key molecular changes invoked prior to, and subsequently to, Httex1 aggregation.

1. Introduction

Protein misfolding and aggregation into amyloids underlies many of the major human neurodegenerative diseases, including Alzheimer's, Parkinson's, Huntington's and motor neuron disease (Sipe et al., 2016). In these diseases, the appearance of macroscopic intracellular protein aggregates known as inclusions form as a hallmark of disease development. Huntington's disease (HD) is one of the classic examples of the amyloid diseases involving intracellular protein aggregating into inclusions. HD involves an expansion of a CAG trinucleotide repeat sequence, encoding polyglutamine (polyQ), within exon 1 of huntingtin (Htt) beyond the disease threshold of 35 repeats (MacDonald et al., 1993). The polyQ expansion leads to a greatly enhanced capacity of the Htt exon 1 (Httex1) domain to aggregate into amyloid fibrils in vitro (Scherzinger et al., 1999) and for N-terminal fragments similar in length to Httex1 to form microscopic inclusion bodies in pathology (DiFiglia et al., 1997; Kazantsev et al., 1999). The link to disease from polyQ-expanded Httex1 has been well documented. Namely, the transgenic expression of polyQ-expanded Httex1 is sufficient to produce a HD-like pathology in rodent and primate models (Davies et al., 1997; von Horsten et al., 2003; Yang et al., 2008) and confers toxicity in cell culture models as well (Arrasate et al., 2004). As such Httex1 has been used extensively to model HD biology.

An outstanding question remains as to the role of aggregation in

Abbreviations: PolyQ, Polyglutamine; Htt, Huntingtin; Httex1, Htt Exon 1; HD, Huntington's disease; PulSA, Pulse Shape Analysis; i, Cells With Inclusions; ni, Cells With Diffuse Httex1; RFU, Relative Fluorescence Units; IPA, Ingenuity Pathway Analysis; FDR, False Discovery Rate; WT, Wild-Type; GFP, Green Fluorescent Protein; ROI, Region Of Interest

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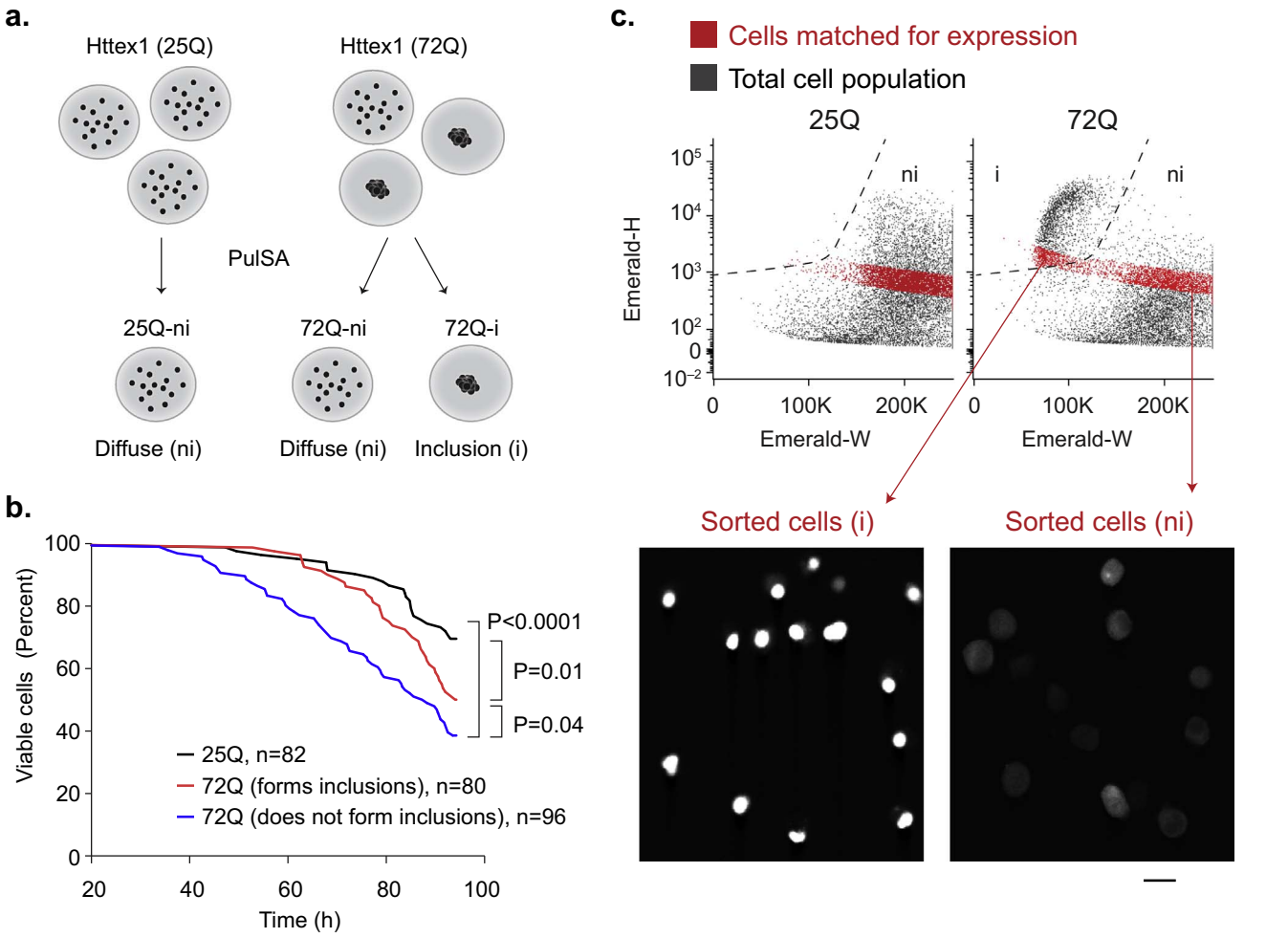

Fig. 1. Strategy to separate cells into populations enriched with polyQ-expanded Httex1 in distinct aggregation states. a) Pulse Shape Analysis (PulSA) by flow cytometry can separate cell populations heterogeneous in aggregation state. b) Neuro2a cells that form inclusions live longer than those that do not. Shown are Kaplan-Meier survival curves tracked by longitudinal imaging of Httex1-Emerald fusions from 20 h after transfection. *P*-values refer to log-rank test (Mantel Cox) on survival curves. c) Flow cytograms showing the PulSA gating strategy to sort the mixed cell populations for analysis by whole transcriptome analysis. Shown are gates for i and ni and for matched expression (red; Further details in Fig. S1). The images show representative cells collected by sorting flow cytometry and imaged for GFP fluorescence by confocal microscopy. Scale bar, 20 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pathogenesis. Evidence increasingly suggests that cellular malfunctioning originates prior to aggregation of Htt into the inclusion body – indeed formation of inclusions seems to aid survival (reviewed in Arrasate and Finkbeiner, 2012; Hatters, 2012). Therefore, soluble polyQ-expanded Httex1 states (including monomers and oligomers) may directly confer cellular toxicity and that toxicity could become nullified as the soluble states are sequestered into the visible aggregates. The mechanisms mediating the origins of cellular toxicity and inclusion assembly remain to be properly elucidated.

Until recently, it has not been straightforward to decipher the cellular changes arising from polyQ-expanded Httex1 as it shifts from soluble states into the inclusion body because cell populations contain mixtures of cells enriched with Httex1 in different aggregation states. This heterogeneity prevents a clean delineation of effects attributable to mutation from aggregation state.

We previously developed a flow cytometry based method (Pulse Shape Analysis: PulSA) to separate cells expressing polyQ-expanded Httex1 into two major groups: those enriched with Httex1 in inclusions (i) and those enriched with soluble Httex1 states (predominantly monomer) (ni) (Ramdzan et al., 2012) (Fig. 1a). This methodology provides a unique opportunity to explicitly understand how aggregation state of Httex1 (or indeed any mutant protein that forms an inclusion) relates to molecular dysfunction. Therefore, in this study, we sought to comprehensively determine how the transcriptome is altered by Httex1 aggregation in a cell culture model that is both sensitive to mutant Httex1 toxicity and which can be robustly controlled for covariates such as time and expression level. We show that Httex1 provides the most profound changes to the transcriptome prior to aggregation and that the toxicity can be accounted for by a sustained shutdown of baseline CREB signalling from soluble Httex1.

2. Materials and methods

2.1. DNA vectors and constructs

pT-REx vectors (Life Technologies) encoding Httex1 with 25Q or 72Q polyQ sequence lengths were prepared as described previously (Ramdzan et al., 2012; Ramdzan et al., 2010). pGW1-based Httex1-mCherry constructs were prepared as described previously (Tsvetkov et al., 2010).

2.2. Cell culture

Neuro2a cells, obtained originally from the American Type Culture Collection, were maintained in Optimem medium (Life Technologies) supplemented with 10% v/v FCS, 1 mM glutamine, 200 U/ml penicillin and 200 μg/ml streptomycin in a humidified incubator with 5% v/v atmospheric CO_2. Neuro2a cells were transiently transfected with the