

Short reports

Wild type huntingtin reduces the cellular toxicity of mutant huntingtin in mammalian cell models of Huntington's disease

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Abstract

Objectives—Recent data suggest that wild type huntingtin can protect against apoptosis in the testis of mice expressing full length huntingtin transgenes with expanded CAG repeats. It is not clear if this protective effect was confined to particular cell types, or if wild type huntingtin exerted its protective effect in this model by simply reducing the formation of toxic proteolytic fragments from mutant huntingtin.

Methods—We cotransfected neuronal (SK-N-SH, human neuroblastoma) and non-neuronal (COS-7, monkey kidney) cell lines with HD exon 1 (containing either 21 or 72 CAG repeats) construct DNA and either full length wild type huntingtin or pFLAG (control vector).

Results—Full length wild type huntingtin significantly reduced cell death resulting from the mutant HD exon 1 fragments containing 72 CAG repeats in both cell lines. Wild type huntingtin did not significantly modulate cell death caused by transfection of HD exon 1 fragments containing 21 CAG repeats in either cell line.

Conclusions—Our results suggest that wild type huntingtin can significantly reduce the cellular toxicity of mutant HD exon 1 fragments in both neuronal and non-neuronal cell lines. This suggests that wild type huntingtin can be protective in different cell types and that it can act against the toxicity caused by a mutant huntingtin fragment as well as against a full length transgene.

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Keywords: Huntington's disease; huntingtin; apoptosis

Huntington's disease (HD) is an autosomal dominant neurodegenerative condition associated with abnormal movements, cognitive deterioration, and psychiatric symptoms. The causative mutation is a (CAG)_n trinucleotide repeat expansion of more than 35 repeats,

which is translated into an abnormally long polyglutamine tract in the huntingtin protein.^{1,2}

HD is a member of a family of neurodegenerative diseases caused by CAG/polyglutamine expansions, which include spinobulbar muscular atrophy (SBMA), spinocerebellar ataxias (SCA) types 1, 2, 3, 6, and 7, and dentatorubral-pallidoluysian atrophy. All diseases are dominantly inherited (except for SBMA, which is X linked). In all cases, age at onset correlates inversely with repeat number.² The polyglutamine expansion mutation causes disease by conferring a novel deleterious function on the mutant protein and the severity correlates with increasing CAG repeat number and expression levels in transgenic mice and in cell culture models.^{3,4}

While each of these diseases is associated with specific regions of neurodegeneration (which in some cases overlap), they are probably caused by similar pathological processes. A hallmark of many of these diseases, including HD, spinobulbar muscular atrophy (SBMA), dentatorubral-pallidoluysian atrophy (DRPLA), and spinocerebellar ataxias (SCA) types 1, 2, 3, 6, and 7, is the development of intracellular protein aggregates (inclusions) in the vulnerable neurones. However, the pathogenic role of these aggregates is the subject of vigorous debate.⁵

The function of wild type huntingtin is unclear. However, Rigamonti *et al*⁶ recently showed that wild type huntingtin can protect CNS cells from a variety of apoptotic stimuli, including serum withdrawal, stimulation of death receptors, and pro-apoptotic Bcl-2 homologues. We were interested to test if wild type huntingtin protected against the toxicity of polyglutamine expansion mutations. While the experiments in the current paper were in progress, Leavitt *et al*⁷ provided *in vivo* evidence suggesting that wild type huntingtin can protect against the gain of function mutation caused by the expanded polyglutamine tract in mutant huntingtin, using a YAC transgenic mouse model. This study specifically studied apoptosis in the testis, an organ with very high huntingtin expression.⁸ It is relevant to test if these protective effects are seen

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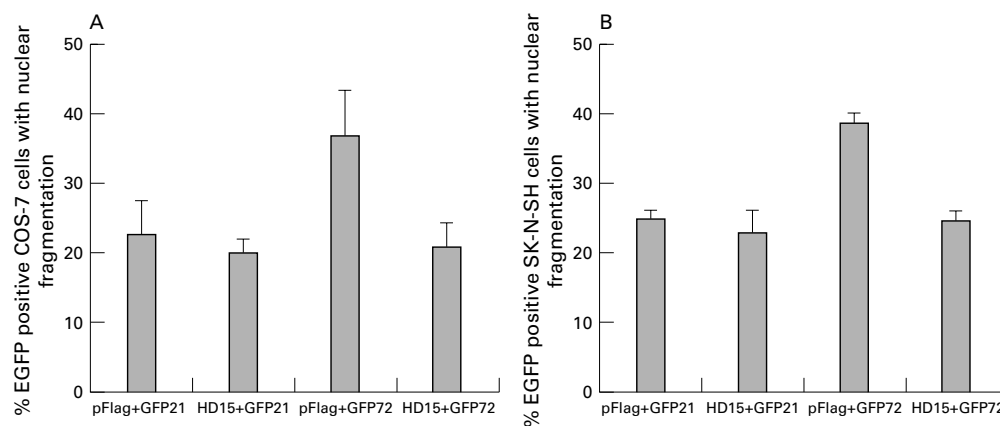


Figure 1 Percentage of EGFP positive COS-7 cells (A) and SK-N-SH cells (B) with fragmented nuclei in cotransfection experiments where full length wild type huntingtin (HD15) or empty vector (pFlag) were transfected with EGFP-huntingtin exon 1 fragments with 21 (GFP21) or 72 CAG (GFP72) repeats. Means (and SEM) from two experiments in triplicate are shown.

in other cell types, as the testis may require high levels of huntingtin to protect against apoptotic insults.⁹ In addition, Leavitt *et al*⁷ showed that wild type huntingtin was protective against full length mutant huntingtin. Full length mutant huntingtin is likely to be cleaved to form a toxic fragment that ultimately mediates the effects of this mutation.¹⁰ Thus, wild type huntingtin may be protecting by reducing the cleavage of mutant full length huntingtin, as opposed to attenuating the apoptosis caused by the toxic fragment.

Methods

We have tested the protective effects of full length huntingtin against the toxicity of polyglutamine expansions in neuronal (SK-N-SH, human neuroblastoma) and non-neuronal (COS-7, monkey kidney) cell lines. We studied the effects of wild type huntingtin on cells expressing exon 1 huntingtin fragments with varying CAG repeat lengths tagged at the N-terminus with enhanced green fluorescent protein EGFP.¹¹ These fragments do not appear to undergo cleavage events, resulting in the formation of stable products (unpublished data). We cotransfected cells with a 1:6 mass ratio of HD exon 1 (containing either 21 or 72 CAG repeats) construct DNA and either full length wild type huntingtin or pFLAG (control vector), to ensure that all cells expressing HD exon 1 constructs also expressed the appropriate pFLAG/full length wild type huntingtin constructs. We used a total of 2.1 μ g (1.8 μ g + 0.3 μ g per well) of DNA per 3.5 cm dish. Cells were fixed 48 hours post-transfection as described previously.¹¹ The full length wild type huntingtin construct (15 repeats) was kindly provided by Dr Michael Hayden.¹⁰ Immunocytochemical analyses, using a mouse anti-huntingtin antibody to residues 181-810 (Chemicon), showed that cells transfected with this construct, under the experimental conditions described above, had significantly higher expression than untransfected cells (data not shown). The huntingtin exon 1 fragments expressed in pEGFP-C1 have been described previously.¹¹ We analysed between 200 and 300 EGFP expressing cells per slide (blinded) in

multiple, randomly chosen visual fields, to ascertain the proportions of cells forming one or more inclusions and cells with nuclear fragmentation, as an index of cell death.¹¹ Pooled estimates for the changes in inclusion formation and cell death resulting from perturbations assessed in two independent experiments (each done in triplicate) were calculated as odds ratios (OR) with 95% confidence intervals, determined by unconditional logistic regression analysis, using the general log linear analysis option of SPSS10 software (SPSS, Chicago).

Results

Full length wild type huntingtin significantly reduced cell death resulting from the mutant HD exon 1 fragments containing 72 CAG repeats (GFP72) in SK-N-SH cells ($p < 0.0001$, OR=0.52, 95% CI 0.44-0.62) and in COS-7 cells ($p < 0.0001$, OR=0.45, 95% CI 0.48-0.67) (fig 1). Wild type huntingtin did not significantly modulate cell death caused by transfection of HD exon 1 fragments containing 21 CAG repeats in either cell line (SK-N-SH cells: $p > 0.05$, OR=0.9, 95% CI 0.74-1.10; COS-7 cells: $p > 0.05$, OR=0.86, 95% CI 0.71-1.04). In COS-7 cells, inclusion formation caused by the mutant HD exon 1 fragments was increased in the presence of wild type full length huntingtin from a mean of 24% to 34% of GFP expressing cells ($p < 0.0001$, OR=1.62, 95% CI 1.37-1.93). In SK-N-SH cells expressing mutant huntingtin exon 1 fragments, overexpression of wild type huntingtin had no significant effect on inclusion formation (10.2% with pFLAG *v* 9.6% with full length huntingtin, $p > 0.05$). These data suggest that wild type huntingtin does not reduce polyglutamine induced cell death by reducing inclusion formation.

Discussion

In summary, our results suggest that wild type huntingtin can significantly reduce the cellular toxicity of mutant HD exon 1 fragments in both neuronal and non-neuronal cell lines, and complement the data presented by Leavitt *et al*⁷ in the testis. This suggests that wild type

huntingtin can be protective in different cell types and that it can act against the toxicity caused by a mutant huntingtin fragment as well as against a full length transgene.

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