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Research Report

Identification of novel polyglutamine-expanded aggregation species in spinal and bulbar muscular atrophy

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ABSTRACT

Polyglutamine-repeat disorders are part of a larger family of neurodegenerative diseases characterized by protein misfolding and aggregation. In spinal and bulbar muscular atrophy (SBMA), polyglutamine expansion within the androgen receptor (AR) causes progressive debilitating muscular atrophy and lower motor neuron loss in males. Although soluble polyglutamine-expanded aggregation species are considered toxic intermediates in the aggregation process, relatively little is known about the spectrum of structures that are formed. Here we identify novel polyglutamine-expanded AR aggregates that are SDS-soluble and bind the toxicity-predicting antibody 3B5H10. Soluble, 3B5H10-reactive aggregation species exist in low-density conformations and are larger by atomic force microscopy, suggesting that they may be less compact than later-stage, insoluble aggregates. We demonstrate disease-relevance *in vivo* and draw correlations with toxicity *in vitro*.

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1. Introduction

Spinal and bulbar muscular atrophy (SBMA) is a slowly progressive X-linked disorder caused by an expanded polyglutamine tract in the androgen receptor (AR) protein. Clinically, SBMA patients display signs of lower motor neuron loss and muscle atrophy. The disease manifestations occur primarily in males, due to the dependence of the disease process on ligand binding of either testosterone or dihydrotestosterone (DHT) to the mutant AR (Chevalier-Larsen et al., 2004; Katsuno et al., 2002; Takeyama et al., 2002; Walcott and Merry, 2002). Several additional native functions of the androgen receptor are required for toxicity in model systems, including nuclear localization, an interdomain amino-/carboxyl (N-/C-) interaction, and AR acetylation (Montie et al., 2009, 2011; Nedelsky et al., 2010; Orr et al., 2010). A hallmark

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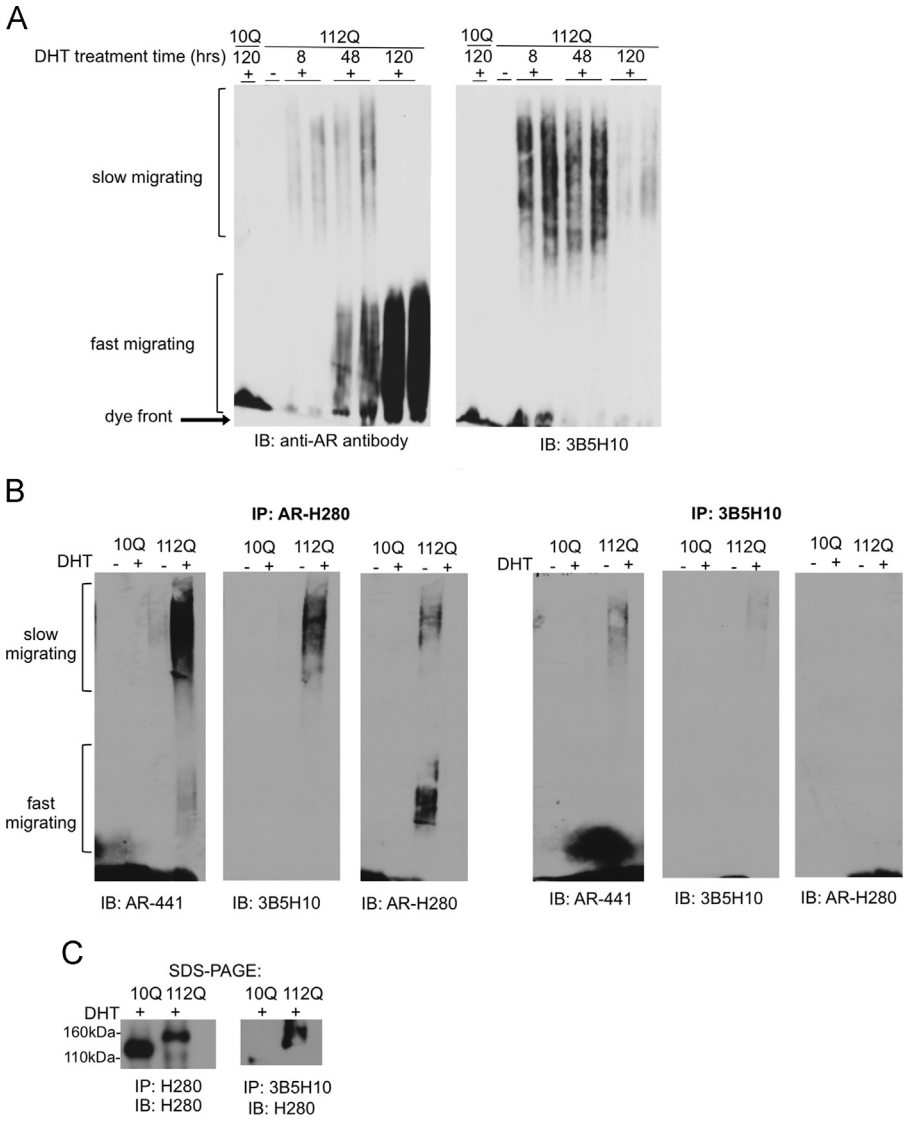

Fig. 1 – Soluble, 3B5H10-reactive polyglutamine-expanded AR aggregation species precede the formation of nuclear inclusions. (A) DHT-treated PC12 cells expressing AR10Q or AR112Q were lysed in 2% SDS and resolved by SDS-agarose gel electrophoresis (SDS-AGE). Two distinct populations (indicated) of AR aggregation species were resolved in a DHT- and polyglutamine-length dependent manner. Slow-migrating species form early in the course of hormone-treatment and bind the toxicity-predicting antibody 3B5H10. (B) PC12 cells expressing AR10Q or AR112Q were treated for 120 h with DHT. Cells were lysed in RIPA and subject to immunoprecipitation with a conformation-specific antibody, 3B5H10, or a pan-AR antibody, AR(H280). Western analysis was performed with AR(441), which detects the full-length protein; the blots were then stripped and re-probed with 3B5H10, followed by AR(H280). To evaluate the efficiency of the IP and 10% of each immunoprecipitate was run on SDS-PAGE; levels of monomer were evaluated through western analysis with AR(H280).

pathology of SBMA is the formation of nuclear inclusions – a feature shared with all 9 of the known neurodegenerative diseases caused by polyglutamine expansion, including Huntington's disease (HD) (reviewed by (Orr and Zoghbi, 2007)). This suggests that common features may exist between misfolded polyglutamine-expanded protein species and cellular toxicity.

We previously reported that motor neurons expressing polyglutamine-expanded AR die before detectable inclusion body formation (Heine et al., 2015), implicating a role for pre-inclusion polyglutamine-expanded AR aggregation species in toxicity. In Huntington's disease, inclusion bodies are thought to promote survival through a process or processes that involve the sequestration of misfolded polyglutamine-expanded huntingtin (Arrasate et al., 2004; Miller et al., 2011) and the restoration of the ubiquitin-proteasome system (Mitra et al., 2009). To this end, the protein level, conformation, and stability of pre-inclusion huntingtin species are thought to predict toxicity (Miller et al., 2011; Mitra et al., 2009; Tsvetkov et al., 2014).

The mechanisms by which misfolded proteins cause toxicity remain unclear. Prevailing hypotheses include enhanced functions of the native protein (Duvick et al., 2010; Nedelsky et al., 2010), aberrant protein interactions (Ratovitski et al., 2012; Steffan