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Impaired ATF6α processing, decreased Rheb and neuronal cell cycle re-entry in Huntington's disease

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ABSTRACT

The endoplasmic reticulum-stress response is induced in several neurodegenerative diseases and in cellular models of Huntington's disease. However, here we report that the processing of ATF6α to its active nuclear form, one of the three branches of endoplasmic reticulum-stress activation, is impaired in both animal models and Huntington's disease patients. ATF6α has been reported to be essential for the survival of dormant tumour cells that, like neurons, are arrested in the G0–G1 phase of the cell cycle. This effect is mediated by the small GTPase Rheb (Ras-homologue enriched in brain). Our results suggest that the ATF6α/Rheb pathway is altered in Huntington's disease as the decrease in ATF6α processing is accompanied by a decrease in the accumulation of Rheb. These alterations correlate with the aberrant accumulation of cell cycle re-entry markers in post-mitotic neurons which is accompanied by death of a subset of neurons.

Introduction

Huntington's disease (HD) is a neurodegenerative disorder caused by the expansion of a CAG triplet encoding a polyglutamine (polyQ) sequence in the amino-terminal end of the huntingtin protein (The Huntington's Disease Collaborative Research Group, 1993). The pathology is characterized by striatal atrophy and the formation of aberrant intraneuronal inclusion bodies composed of mutant huntingtin in varying degrees of aggregation (Davies et al., 1997).

Endoplasmic reticulum-stress (ER-stress) is induced in cells in response to the accumulation of misfolded proteins. Given the presence of aberrant protein aggregates in many neurodegenerative diseases, considerable attention has focussed on exploring the potential role of ER-stress in some of these pathologies (Carnemolla et al., 2009; Duennwald and Lindquist, 2008; Matus et al., 2008; Nakayama et al., 2008; Paschen and Mengesdorf, 2005; Reijonen et al., 2008). Vertebrates express three ER-resident transmembrane proteins that act as sensors of ER-stress: PERK, IRE1α and ATF6α. In conditions of ER-stress these proteins initiate a series of responses (Fig. 1A) which include the activation of genes encoding proteins mediating protein folding (ER-chaperones), the enhancement of the degradation of misfolded proteins and the reduction of protein synthesis. Failure to re-establishing cellular homeostasis results in eventual cell death by apoptosis (Patil and Walter, 2001).

The ER-stress sensor ATF6α is primarily an ER transmembrane protein. Induction of ER-stress however initiates increased trafficking of full-length ATF6α (fl-ATF6α) from the ER to the Golgi. There, fl-ATF6α is sequentially cleaved by two Golgi proteases S1P and S2P to release the cytosolic N-terminal ATF6α region (Nt-ATF6α) that acts as a transcription factor of the basic leucine zipper (bZIP) family upon migration to the nucleus (Haze et al., 1999; Ye et al., 2000).

Recently, ATF6α has been reported to be critical for the survival of dormant tumour cells (Schewe and Aguirre-Ghiso, 2008). These cells are arrested in the G0–G1 phase of the cell cycle. The survival effect of ATF6α is mediated by Rheb (Ras-homologue enriched in brain), an activator of mTOR (mammalian target of rapamycin) (Manning and Cantley, 2003), with genetic ablation of ATF6α in these cells resulting in apoptosis.

In this paper we report that the processing of ATF6α, a canonical sensor of ER-stress, is impaired in both animal models of the disease as well as in the brains of HD patients. This impairment is manifested in a decreased accumulation of Nt-ATF6α and a concomitant accumulation of the fl-ATF6α. Accordingly, markers of ER-stress are

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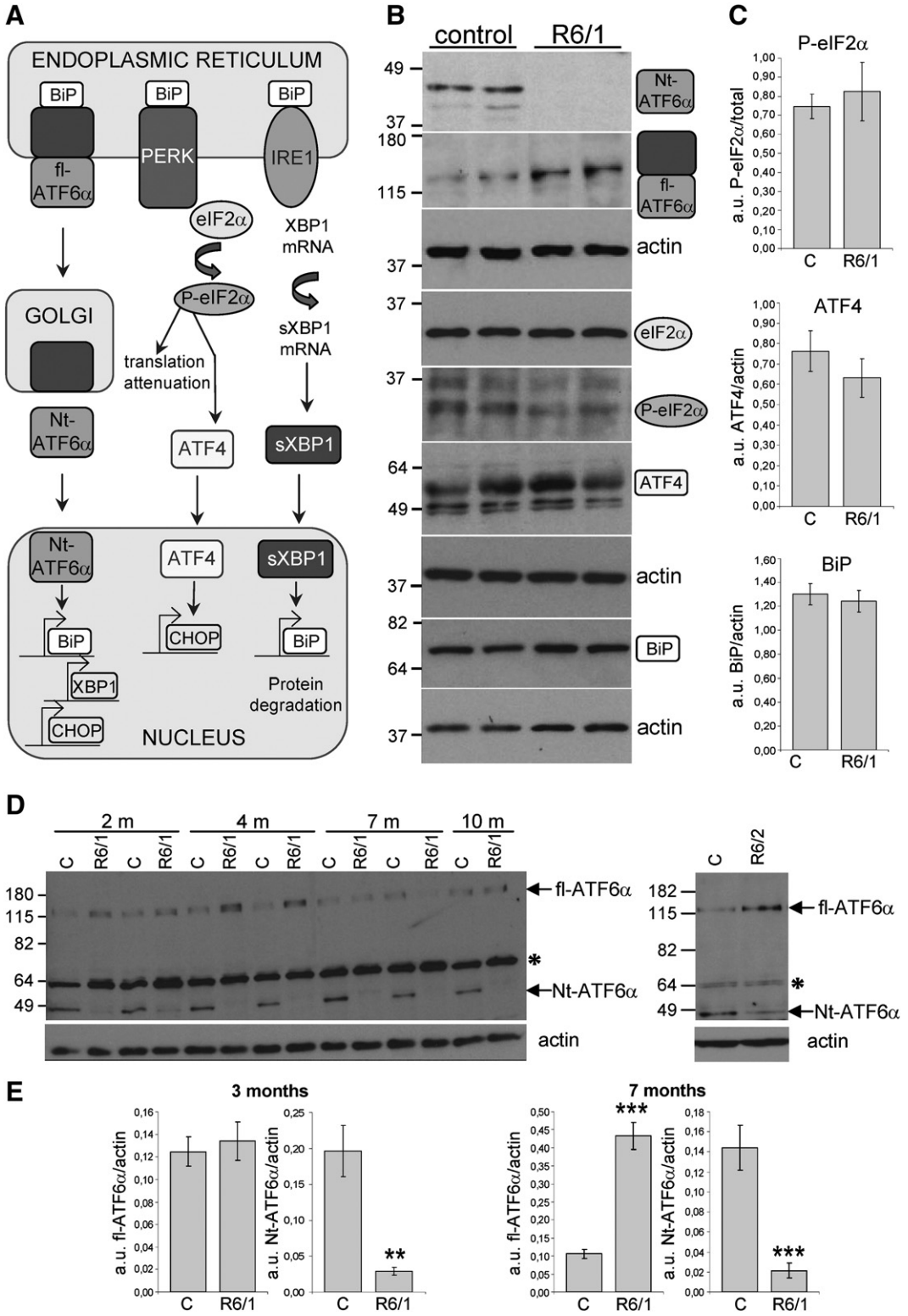

Fig. 1. The processing of ATF6α is impaired in HD animal models. (A) Schematic representation of the three ER-stress branches. In conditions of ER-stress a variety of downstream responses are activated (representative ones are shown). ATF6α exits the ER to reach the Golgi where it is processed by two Golgi proteases (S1P and S2P). The cytoplasmic N-terminal domain (Nt-ATF6α) is a transcription factor that migrates to the nucleus where it activates the productions of ER-chaperones (i.e. BiP), XBP1 or CHOP. PERK phosphorylates the eukaryotic translation initiation factor eIF2α. Protein synthesis is generally reduced but there is an increased translation of mRNAs containing small open reading frames in their 5' untranslated regions. This is the case of ATF4 transcription factor that consequently activates the production of downstream genes. IRE1 initiates the non-conventional splicing of XBP1 mRNA. The spliced form encodes a transcription factor that controls the production of ER chaperones and ER-related protein degradation (ERAD) proteins. (B) The accumulation of ER-stress markers was explored in 7-month-old control and R6/1 animals. The levels of actin served as a protein loading control. In the case of P-eIF2α the total amount of eIF2α protein served as control. The absence of Nt-ATF6α in R6/1 animals correlates with increased levels of fl-ATF6α. (C) The accumulation of P-eIF2α, ATF4 and BiP was quantified at 7 months using 7 control and 7 R6/1 animals. The quantification shows no significant difference in the levels of any of the proteins (respectively $p = 0.649$, $p = 0.360$ and $p = 0.647$) between control and R6/1 animals. (D) Western-blot of R6/1 animals at different ages, R6/2 animals at the age of 3 months and controls using an anti-ATF6α antibody that recognizes both Nt-ATF6α and fl-ATF6α. (*) indicates an unspecific band recognized by the antibody. Actin levels are shown below each panel. (E) The accumulation of fl- and Nt-ATF6α was quantified at two representative ages, 3 months (pre-symptomatic) and 7 months (symptomatic), using 7 control and 7 R6/1 animals in each case. The decrease in the accumulation of Nt-ATF6α was significant both at 3 months ($p = 0.01$) and 7 months ($p < 0.001$). The increase in the accumulation of fl-ATF6α was not significant at the age of 3 months ($p = 0.750$) but was highly significant at 7 months ($p < 0.001$).