Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

Signature changes in ubiquilin expression in the R6/2 mouse model of Huntington's disease

Nathaniel Safren[a,b], Lydia Chang[b], Kristina M. Dziki[b], Mervyn J. Monteiro[a,b,c,*]

[a]Neuroscience Graduate Program, University of Maryland School of Medicine, Baltimore, MD 21201, United States
[b]Center for Biomedical Engineering and Technology, University of Maryland School of Medicine, Baltimore, MD 21201, United States
[c]Department of Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, 20 North Pine Street, Baltimore, MD 21201, United States

ARTICLE INFO

Article history:
Accepted 3 December 2014
Available online 12 December 2014

Keywords:
Ubiquilin
Huntington's disease
Inclusions
Brain
Ubiquitin

ABSTRACT

Ubiquilin proteins have been implicated in the cause and the pathology of neurodegenerative diseases. In the R6/2 mouse model of Huntington's disease (HD), ubiquilin levels decline during disease progression. Restoration of their levels by transgenic expression of ubiquilin-1 extends survival. Here we provide a comprehensive assessment of the expression and localization of all four ubiquilin proteins in both normal and R6/2-affected mice brains, using antibodies specific for each protein. Ubiquilin-1, 2 and 4 proteins were detected throughout the brain, with increased expression seen in the hippocampus and cerebellum. Ubiquilin-3 expression was not detected. All three ubiquilins expressed in the brain were found in Htt inclusions. Their expression changed during development and disease. Ubiquilin-1 and ubiquilin-2 protein levels decreased from 6 to 18 weeks of mouse development, independent of disease. Ubiquilin-1 and ubiquilin-4 protein levels also changed during HD disease progression. Ubiquilin-4 proteins that are normally expressed in the brain were lost and instead replaced by a novel 115 kDa higher molecular weight immunoreactive band. Taken together, our results demonstrate that all ubiquilin proteins are involved in HD pathology and that distinct changes in the signature of ubiquilin-4 expression could be useful for monitoring end-stage of HD disease.

1. Introduction

Huntington's disease (HD) is a debilitating neurodegenerative disorder caused by a polyglutamine expansion in huntingtin (Htt) protein (1993). There is an inverse correlation between the length of the polyglutamine expansion and age of onset of the disease (Walker, 2007). Longer polyglutamine tracts increase the propensity of mutant Htt protein to aggregate,

*Corresponding author at: Center for Biomedical Engineering and Technology, Room S642, University of Maryland School of Medicine, 20 North Pine Street, Baltimore, MD 21201, United States. Fax: +410 706 8184.
E-mail address: monteiro@umaryland.edu (M.J. Monteiro).

http://dx.doi.org/10.1016/j.brainres.2014.12.008
0006-8993/

forming ubiquitin-positive inclusion bodies that are a pathological hallmark of HD (Finkbeiner, 2011). Several reports indicate that Htt inclusions contain ubiquilin, a protein that functions in protein clearance through the proteasome and autophagy pathways (Doi et al., 2004; Mori et al., 2012; Rutherford et al., 2013). Interestingly, in R6/2 mice, which recapitulate many features of HD, ubiquilin proteins are not only present in Htt inclusions, but their levels decline progressively during disease progression (Safren et al., 2014). Restoration of ubiquilin levels by transgenic overexpression of ubiquilin-1 extends survival of R6/2 mice suggesting the decline in ubiquilin levels affects disease (Safren et al., 2014).

Both humans and mouse contain four ubiquilin genes (UBQLN1 to 4), each encoding a separate protein. The four proteins share an N-terminal ubiquitin-like domain (UBL) and C-terminal ubiquitin-associated domain (UBA), but differ from each other due to insertions and deletions in their central domain (Mah et al., 2000; Wu et al., 1999, 2002; Davidson et al., 2000). The domain structure of the proteins is typical of shuttle factors that bind and deliver polyubiquitinated proteins to the proteasome (Elsasser and Finley, 2005). Indeed ubiquilin proteins not only function in proteasome degradation, but also in autophagy (Kleijnen et al., 2003, 2000; Ko et al., 2004; Lim et al., 2009; N'Diaye et al., 2009; Rothenberg and Monteiro, 2010; Rothenberg et al., 2010).

Genetic mutations in UBQLN1, 2 and 4 genes have all been linked to different neurodegenerative diseases (Deng et al., 2011; Fahed et al., 2014; Gonzalez-Perez et al., 2012; Yan et al., 2013). It is possible that the mutations in each ubiquilin gene cause a different spectrum of disease due to variability in the expression of the genes throughout the nervous system. However, the distribution of each ubiquilin protein in the brain is not known. Here we used antibodies specific for each of the four ubiquilins to determine their expression patterns in mouse brain. We also used the antibodies to determine whether all ubiquilins colocalize with Htt inclusion bodies in R6/2 mice, as this was unknown. We further examined whether expression of each ubiquilin changes with disease progression.

2. Results

2.1. *Characterization of antibodies that discriminate each of the four ubiquilin proteins in mouse*

In order to assess the profile and distribution of ubiquilin expression in normal and HD-afflicted mouse brains we screened ubiquilin antibodies from commercial sources and the ones we had generated to identify those that were specific for each of the four ubiquilin gene products expressed in mammals. To establish their specificity, each of the four different ubiquilin isoforms was expressed as a GFP-fusion protein in mouse NB2a neuroblastoma cells and HeLa cells (Fig. 1). Protein lysates from the transfected cells, and the mock-transfected control, were probed with the antibodies to see which, and how many GFP-ubiquilin-fusion proteins, were recognized by the ubiquilin antibodies. For these tests,

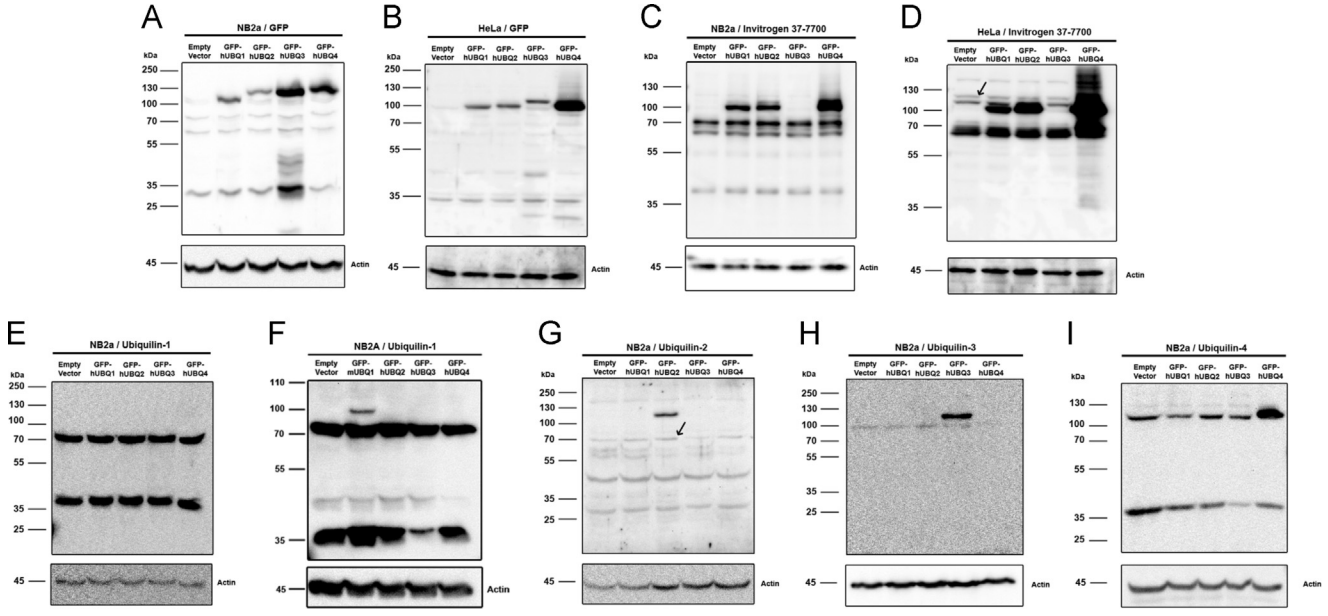

Fig. 1 – Specificity of ubiquilin antibodies. Lysates from HeLa and NB2A mouse neuroblastoma cells transfected with GFP-ubiquilin cDNAs. Successful expression of human ubiquilin-1 (hUBQ1), 2, 3 and 4 fusion proteins in (A) NB2A cells and (B) HeLa cells. (C and D) The Invitrogen 37-7700 antibody recognizes both mouse and human ubiquilin 1 and 2, as well as human ubiquilin-4. (D) In HeLa cell lysates this antibody also recognizes endogenous ubiquilin-4 (marked with an arrow). (E) The PA1 Ubiquilin-1 antibody fails to recognize human ubiquilin-1. (F) Lysates from NB2A cells transfected with a construct encoding GFP-mouse-ubiquilin-1 (mUBQ1) indicates the PA1 ubiquilin-1 antibody does specifically recognize mouse ubiquilin-1. Endogenous ubiquilin-1 runs as two distinct bands, one at 70 kDa and one at 35 kDa. (G) UMY75 specifically recognizes the transfected ubiquilin-2 product. The arrow highlights a band predicted to be the endogenous ubiquilin-2 protein. (H) UMY78 specifically recognizes ubiquilin-3. (I) ARP57-355 specifically binds ubiquilin-4.