57  Full length huntingtin is not detected in intranuclear inclusions in Huntington's disease brain

Philip Thomas1, Fiona Wilkinson1, Nguyen thi Man1, Peter S Harper1, James W Neal1, Glenn E Morris1 and A Lesley Jones1
1 Institute of Medical Genetics 2 Dept of Pathology, University of Wales College of Medicine, Cardiff CF4 4XN and 3. MRC Biochemistry Group, NE Wales Institute, Plas Coch, Wrexham, LL11 2AW, UK.

Huntington's disease is an incurable autosomal dominant neurodegeneration, associated with an expanded CAG repeat in the 5' coding region of a large gene, which gives rise to an expanded polyglutamine sequence in the protein, huntingtin[1]. The normal range of this repeat is up to 35 CAG and expansions of greater than 36 CAG give rise to the disease [2]. Recent evidence has shown the presence of huntingtin immunoreactive neuronal intranuclear inclusions (NIIs) in mice transgenic for exon 1 of huntingtin with expanded repeats (115-150) [3] and in HD brain [4]. Other diseases associated with polyglutamine expansions show similar intranuclear inclusions [5, 6]. It remains unclear whether the NIIs seen in the diseases are the pathological event leading to neuronal degeneration, a consequence of some other event also leading to neuronal degeneration or a defence mechanism by which neurons attempt to sequester polyglutamine tracts. It has been observed that polyglutamine within truncated rather than full length proteins shows increased toxicity [7] and that a polyclonal antibody that detects amino acids 585-725 of huntingtin does not stain NIIs in HD brain [4]. We have generated monoclonal antibodies (mAbs) to huntingtin-GST fusion proteins, against amino acids 997-1276, 1844-2131 and 2703-2911 of huntingtin [8]. Three mAbs, 3E10 (997-1276), 4E10 (1844-2131) and 8A4 (2703-2911) have been characterised and used in a survey of huntingtin immunoreactivity in Huntington's disease (HD) and normal brain, to analyse the presence of huntingtin epitopes C-terminal to polyglutamine in NIIs.

Formalin fixed paraffin embedded serial sections from four pathologically and molecularly characterised cases of HD and a similar number of control cases were used (post-mortem intervals 24-48h). We concentrated on caudate as the area most affected pathologically, cortex, which is also known to degenerate in later stages of the disease, and cerebellum, which is not known to be affected in HD. Figures 1a and b show the presence of NIIs in the brains used detected by ubiquitin staining (monoclonal anti ubiquitin MAB1510, Chemicon). We found clear evidence of inclusions particularly in the lower cortical layers. The cases used were Vonsattel grades 3/4 and thus the caudate was reduced to a rim of tissue with few neurons and although relatively few NIIs were seen in comparison with the cortex, there were many densely-staining ubiquitin positive areas which were not visible in normal tissue (Fig 1b). The three mAbs used all gave similar patterns of immunoreactivity with most dense staining in neuronal tissue, particularly in large pyramidal neurons in the cortex (Fig 1 c-e). Uniform staining of neuronal cytoplasm was commonly seen with little evidence of any nuclear staining (Fig 1 c-e) and the pattern of immunoreactivity was indistinguishable between HD and normal cortex (data not shown). These patterns of immunoreactivity are similar to those observed previously in human, rat and monkey brain [9-11]. Interestingly, all the MABs in HD caudate showed distinct astrocytic immunoreactivity which has been observed previously (Fig 1f) [12]. No evidence of NII immunoreactivity was seen with any of these mAbs in cortex or caudate, although these were serial sections with those showing ubiquitin immunopositive inclusions. Immunoreactivity in the cerebellum was similar in control and HD brain with all the mAbs with marked, relatively uniform staining of the cytoplasm of Purkinje cells. We therefore confirm the conclusion that full length huntingtin protein is not recruited into NIIs of HD [4]. This is in contrast with spinocerebellar ataxia 3 where full length ataxin 3 with an expanded polyglutamine tract does appear to be recruited into NIIs, although it is a much smaller protein [5]. It appears that processing of huntingtin carrying an expanded polyglutamine tract is important in the formation of NIIs.

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References