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Research Report

Characterization of mutant mice that express polyglutamine in cerebellar Purkinje cells

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ABSTRACT

We recently produced transgenic mice that expressed an abnormally expanded polyglutamine (polyQ) specifically in cerebellar Purkinje cells (polyQ mice). The polyQ mice showed inclusion body formation, cerebellar atrophy and severe ataxia. Here we analyzed polyQ mice using immunohistochemistry, immunoelectronmicroscopy and electrophysiology. A diffuse form of polyQ was detected in the nucleus. Interestingly, ubiquitinated large inclusions were located close to, but apparently outside of the soma of Purkinje cells. Infusion of lucifer yellow into Purkinje cells clearly indicated the traffic between the periplasmic inclusions and soma of Purkinje cells. To examine whether the formation of periplasmic inclusions was an active process or a result of cell death, the polyQ mouse cerebellum was immunolabeled for cleaved caspase-3, a marker of apoptosis. Interestingly, no Purkinje cells in P80 polyQ mice immunoreacted with the antibody. The results were substantiated by electrophysiological assay, which showed that P80 Purkinje cells with large periplasmic inclusions were functionally active: excitatory postsynaptic currents (EPSCs) were reliably evoked upon electrical stimulation of parallel fibers (PFs) or climbing fibers (CFs), and current injection into Purkinje cells generated action potentials; however, the frequency of action potentials in response to various volumes of current injection was consistently lower in polyQ mice than in wild-type animals, and aberrant innervation by multiple CFs was detected in polyQ mouse Purkinje cells. These results suggest that Purkinje cells with periplasmic inclusions were not apoptotic, but their functions were substantially impaired, which could contribute to the severe ataxic phenotype.

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Abbreviations: CF, climbing fiber; EPSC, excitatory postsynaptic current; HA, hemagglutinin; P, postnatal day; PF, parallel fiber; polyQ, polyglutamine; PPD, paired-pulse depression; PPF, paired-pulse facilitation; SCA, spinocerebellar ataxia

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1. Introduction

The cerebellum is critically involved in motor coordination and motor learning (Ito, 2002). Cerebellar Purkinje cells, large inhibitory neurons, develop a well-ramified dendritic tree that forms synapses with parallel fibers, axons of cerebellar granule cells, climbing fibers, axons of neurons in the inferior olivary complex and inhibitory interneurons, such as stellate, basket and Golgi cells. Purkinje cells integrate those signals and output to neurons in deep cerebellar nuclei. Purkinje cells thus play critical roles as final integrators of cortical information in the regulation of motor coordination and motor learning. Although important for cerebellar functions, Purkinje cells are vulnerable and easily damaged by slight insults (Sarna and Hawkes, 2003): the functional deficit of Purkinje cells is directly associated with impaired motor control ability.

Polyglutamine diseases are a growing family of dominantly inherited neurodegenerative disorders caused by abnormal expansion of the polyglutamine stretch. This group now includes Huntington's disease, spinobulbar muscular atrophy, dentatorubropallidoluysian atrophy, and spinocerebellar ataxia (SCA) types 1, 2, 3, 6, 7, and 17 (Orr and Zoghbi, 2007). Expansion of polyglutamine repeats alters the conformation, or results in the misfolding, of disease-associated protein, thereby conferring a toxic gain of function that is selectively deleterious to neurons (Sato et al., 1999; Yoshizawa et al., 2000). Histopathological examinations of the postmortem brains of patients with polyglutamine disease revealed degeneration and loss of neuronal cells in the spinal cord and selective brain regions, including the cerebellum, brainstem and basal ganglia (Durr et al., 1996; Fowler, 1984; Sudarsky and Coutinho, 1995). Neuronal intranuclear inclusions are detected in patients with SCA3 and Huntington's disease and are considered to be a common feature causing polyglutamine-mediated neuronal death (Paulson et al., 1997; Schmidt et al., 1998); however, since, in addition to affected neurons, neuronal intranuclear inclusions were present in spared neurons (Yamada et al., 2001), it is elusive whether the formation of neuronal intranuclear inclusions is required for the manifestation of symptoms in polyglutamine disease.

We recently generated transgenic mice (polyQ mice) expressing an expanded polyglutamine protein (polyQ) in Purkinje cells (Torashima et al., 2008) using a truncated form of human ataxin-3 with 69 CAG triplet repeats (Fig. 1a) (Kawaguchi et al., 1994; Yoshizawa et al., 2000). They showed marked cerebellar atrophy and severe ataxia as early as 3 weeks of age. Immunohistochemical analysis of polyQ mice at postnatal day (P) 25 revealed nuclear localization of the diffuse type of polyQ, but no inclusions in Purkinje cells, suggesting that intranuclear inclusions are not essential for disease manifestation. In our polyQ mice, inclusion bodies started to form at P40, which markedly increased in number and size at P80 (Torashima et al., 2008). Lentivector-mediated expression of CRAG, a protein that facilitates the ubiquitin proteasome pathway (Qin et al., 2006), in Purkinje cells of polyQ mice extensively cleared polyQ aggregates, and eventually resolved ataxia, indicating the strong association of polyQ accumulation in Purkinje cells with the defects of cerebellar function. In this study, we examined Purkinje cells of polyQ mice morphologically and functionally to clarify the mechanisms underlying cerebellar dysfunction and the resulting ataxia.

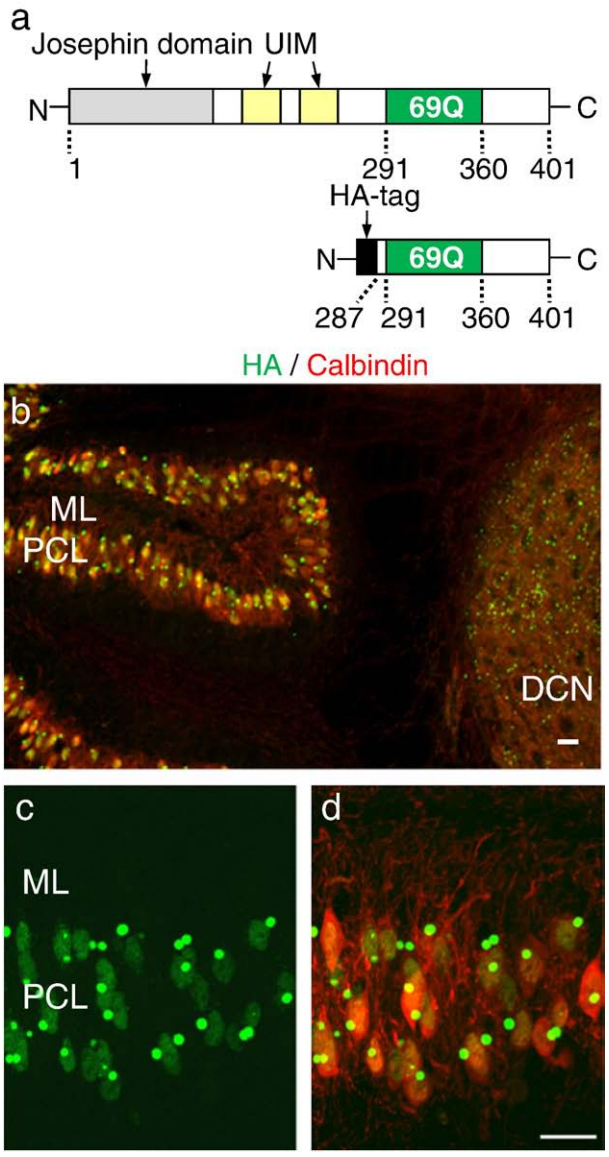

Fig. 1 – The construct used to generate polyQ mice and polyQ accumulation in Purkinje cell. (a) A schema showing wild-type and truncated ataxin-3 protein with an abnormally expanded polyglutamine. The truncated ataxin-3 lacks N-terminal 286 amino acids that contain the ubiquitin proteasome domain (Josephin domain) and ubiquitin interaction motifs (UIMs). Thus, the expressed protein consists of a 69 polyglutamine stretch with only 4 and 42 amino acids at its N-terminus and C-terminus, respectively. HA: hemagglutinin. (b–d) Immunostaining of a sagittal section of P80 polyQ mouse cerebellum with anti-HA (green) and anti-calbindin (red) antibodies. Scale bar, 20 μm (b, d). ML: molecular layer; PCL: Purkinje cell layer; DCN: deep cerebellar nuclei.

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