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Research Report

The anatomy of the cerebellar nuclei in the normal and scrambler mouse as revealed by the expression of the microtubule-associated protein kinesin light chain 3

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ABSTRACT

Conventional kinesin is a motor protein complex including two heavy chains and two light chains (KLC). Junco et al. (Junco, A., Bhullar, B., Tarnasky, H.A. and van der Hoorn, F.A., 2001. Kinesin light-chain KLC3 expression in testis is restricted to spermatids. Biol. Reprod. 64, 1320–1330). recently reported the isolation of a novel KLC gene, *klc3*. In the present report, immunohistochemistry has been used to characterize the expression of KLC3 in the cerebella of normal and *scrambler* (scm) mutant mice. In cryostat sections through the cerebellum of the normal adult mouse immunoperoxidase stained for KLC3, reaction product is deposited in the nuclei and somata of deep cerebellar nuclear neurons. No other structures are stained in the cerebellum. Strong and specific KLC3 expression is observed in the adult cerebellum in all three major cerebellar nuclei—medial, interposed, and lateral. Double immunofluorescence studies reveal that KLC3 immunoreactivity is colocalized with both endosomes and GW bodies. KLC3 immunohistochemistry has been exploited to study the organization of the cerebellar nuclei in *scrambler* mice, in which disruption of the mdab1 gene results in severe foliation defects due to Purkinje cell ectopia, with most Purkinje cells clumped in centrally located clusters. Despite the severe failure of Purkinje cell migration, the cerebellar nuclei appear normal in *scrambler* mutant mice, suggesting that their topography is dependent neither on normal Purkinje cell positioning nor the Reelin signaling pathway.

1. Introduction

The deep cerebellar nuclei (DCN) are the sole output of the cerebellum (e.g., Ito et al., 1970). Classical studies identify four major divisions of the DCN: the medial (fastigial in human), posterior and anterior interposed (globose and emboliform, respectively), and lateral (dentate) nuclei. Cerebellar cortical input to the DCN is supplied via Purkinje cell axons. Anterograde tracing and degeneration studies (Jansen and Brodal, 1940; Armstrong and Schild, 1978a,b) showed that the medial nuclei receive Purkinje cell input from the vermis, the interposed nuclei from the intermediate hemispheres, and the lateral nuclei from the lateral hemispheres. Although mice are a major research model for cerebellar studies, there is no published description of the anatomy of the DCN in mice (as there is for rat, e.g., Voogd et al., 1985). The anatomical

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mapping is also complicated because there have been no DCN-specific antigenic markers. This is not a significant impediment in the normal cerebellum, where the DCN are discrete and separate from the neurons of the cerebellar cortex, but is a problem when exploring the anatomy of cerebella with neuronal ectopia. For example, *scrambler* is a naturally occurring mutation of the gene coding for disabled homolog 1 (disabled-1), which encodes the disabled-1 adaptor protein (Howell et al., 1997; Sheldon et al., 1997). During the formation of the cerebellar cortex, disabled-1 expression is restricted to the Purkinje cell population (Rice et al., 1998). Binding of Reelin to its receptors results in the recruitment of disabled-1 and the phosphorylation of disabled-1 on tyrosine residues (Howell et al., 1997). This activates intracellular signaling pathways that include non-receptor tyrosine and serine/threonine kinases (Bock and Herz, 2003) and trigger Purkinje cell dispersal into a monolayer. Disruption of the *disabled-1* gene (as in *scrambler* mice) results in cerebellar developmental abnormalities, including the failure of normal Purkinje cell dispersal and a complete absence of foliation (Howell et al., 1997; Gallagher et al., 1998; Rice et al., 1998). While the Purkinje cell defects in *scrambler* are well understood, it is not known if DCN architecture is also affected by disruptions of the Reelin→disabled-1 signaling pathway.

Kinesin light chains (KLCs) are components of the kinesin motor complex, which consists of two kinesin heavy chains (KHCs) associated with two KLCs. Kinesins bind to and move along microtubules, powering the transport of macromolecules and organelles (reviewed in Kamal and Goldstein, 2002). Junco et al. (2001) recently reported the isolation of a novel KLC gene, *klc3*, which is expressed in several tissues, including testis and brain (Junco et al., 2001). In testis, KLC3 appears to play a role in the movement of mitochondria towards the forming midpiece in spermatids at late stages of spermatogenesis (Zhang et al., 2004). Here, we report for the first time that in the cerebellum the expression of the microtubule-associated protein KLC3 is restricted to the DCN and exploit this characteristic to provide detailed anatomical maps of the DCN both in normal adult and in *scrambler* mutant mice.

2. Results

2.1. *A brief survey of KLC3 expression in the adult mouse brain*

The expression of the anti-KLC3 immunoreactivity was examined in a variety of adult mouse brain regions (Fig. 1). Most brain regions do not exhibit KLC3 immunoreactivity. Exceptions include the olfactory bulb, where there is moderate KLC3 expression in the glomerular layer and granular layer and intense immunoreactivity in the mitral cells (Fig. 1A), scattered neurons in the cerebral cortex (e.g., Figs. 1B, C), and axonal profiles in the corpus callosum (Figs. 1D, E). KLC3 expression was also seen in cells abutting the 3rd ventricle (Fig. 1F) and piriform cortex (Fig. 1G). In the hindbrain, anti-KLC3 immunocytochemistry deposits strong reaction product in all vestibular nuclei (lateral vestibular—Fig. 1H) and facial nuclei (Fig. 1I). In addition, there was clear staining of the deep cerebellar nuclear complex (DCN: Fig. 2).

2.2. *Cerebellar KLC3 expression in the normal mouse*

Western blots of mouse cerebellum probed with anti-KLC3 showed a band with an apparent molecular weight of 56 kDa (Fig. 2A), consistent with previous reports (Junco et al., 2001). In cryostat sections through the cerebellum of the normal adult mouse immunoperoxidase stained for KLC3, reaction product was deposited in the neurons of the DCN. Other cerebellar neurons, and glial cells, were unreactive (Fig. 2B–D). Seen at higher magnification, reaction product was deposited homogeneously throughout the somata, and, in addition, punctate juxtanuclear, cytoplasmic staining was characteristic. Weakly stained KLC3-immunopositive axons with occasional varicosities are seen in some of the DCN (e.g., Fig. 2D). KLC3 immunoreactivity is present both in large and small DCN neurons (e.g., Fig. 2D inset), although the large neuronal somata are the most prominent. Differences in staining intensity between subnuclei are sometimes observed, but these are inconsistent. In addition to the projection neurons of the DCN, the cerebellum contains two other classes of large neurons—Purkinje cells and Golgi cells. The spatial segregation of these three neuronal types in the normal cerebellum makes them easy to distinguish. However, in contexts of neuronal ectopia, this may not be the case. Therefore, to confirm that Purkinje cells in the mouse cerebellum do not express KLC3, double staining was performed with anti-calbindin (CaBP), a specific marker for all Purkinje cells (e.g., Baimbridge et al., 1982). Fig. 2E shows a transverse section double immunofluorescence stained for KLC3 (red) and CaBP (green). Anti-CaBP uniformly stains the Purkinje cell somata and dendrites. KLC3-immunoreactive DCN neurons were anti-CaBP unreactive. To confirm that Golgi cells do not express KLC3, sections were double-labeled with anti-KLC3 and anti-HNK-1 which stains Golgi cells (e.g., Marzban et al., 2004). HNK-1-immunoreactive Golgi cells did not express KLC3 immunoreactivity (Fig. 2F). To our knowledge, KLC3 is the first antigen whose expression within the cerebellum is restricted to neurons of the DCN.

Previous studies have shown that kinesin can be found bound to numerous cytoplasmic organelles, including mitochondria, synaptic vesicles, coated vesicles (Leopold et al., 1992), secretory granules (Rothwell et al., 1993), early and late endosomes, rough and smooth endoplasmic reticulum, and the Golgi complex (Sato-Yoshitake et al., 1992). Multiple kinesin light chain isoforms are also colocalized with mitochondria (Khodjakov et al., 1998; Zhang et al., 2004) and the Golgi complex (Gyoeva and Gelfand, 1991). To identify the subcellular distribution of KLC3 protein in the cerebellum, we performed double immunofluorescence staining with anti-early endosome 1 (EEA1, Mu et al., 1995), anti-Golgi complex (Fritzler et al., 1993), and anti-GW body antibodies (GW bodies are reactive to autoantigens recognized by immune serum from a patient with ataxic sensory polyneuropathy: Eystathioy et al., 2002; see Jacymiw et al., 2005). Frontal sections double-labeled for anti-KLC3 and anti-EEA1 reveal that most KLC-immunoreactive punctae are colocalized with early