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Progress in pathogenesis studies of spinocerebellar ataxia type 1

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Spinocerebellar ataxia type 1 (SCA1) is a dominantly inherited disorder characterized by progressive loss of coordination, motor impairment and the degeneration of cerebellar Purkinje cells, spinocerebellar tracts and brainstem nuclei. Many dominantly inherited neurodegenerative diseases share the mutational basis of SCA1: the expansion of a translated CAG repeat coding for glutamine. Mice lacking ataxin-1 display learning deficits and altered hippocampal synaptic plasticity but none of the abnormalities seen in human SCA1; mice expressing ataxin-1 with an expanded CAG tract (82 glutamine residues), however, develop Purkinje cell pathology and ataxia. These results suggest that mutant ataxin-1 gains a novel function that leads to neuronal degeneration. This novel function might involve aberrant interaction(s) with cell-specific protein(s), which in turn might explain the selective neuronal pathology. Mutant ataxin-1 interacts preferentially with a leucine-rich acidic nuclear protein that is abundantly expressed in cerebellar Purkinje cells and other brain regions affected in SCA1. Immunolocalization studies in affected neurons of patients and SCA1 transgenic mice showed that mutant ataxin-1 localizes to a single, ubiquitin-positive nuclear inclusion (NI) that alters the distribution of the proteasome and certain chaperones. Further analysis of NIs in transfected HeLa cells established that the proteasome and chaperone proteins co-localize with ataxin-1 aggregates. Moreover, overexpression of the chaperone HDJ-2/HSDJ in HeLa cells decreased ataxin-1 aggregation, suggesting that protein misfolding might underlie NI formation. To assess the importance of the nuclear localization of ataxin-1 and its role in SCA1 pathogenesis, two lines of transgenic mice were generated. In the first line, the nuclear localization signal was mutated so that full-length mutant ataxin-1 would remain in the cytoplasm; mice from this line did not develop any ataxia or pathology. This suggests that mutant ataxin-1 is pathogenic only in the nucleus. To assess the role of the aggregates, transgenic mice were generated with mutant ataxin-1 without the self-association domain (SAD) essential for aggregate formation. These mice developed ataxia and Purkinje cell abnormalities similar to those seen in SCA1 transgenic mice carrying full-length mutant ataxin-1, but lacked NIs. The nuclear milieu is thus a critical factor in SCA1 pathogenesis, but large NIs are not needed to initiate pathogenesis. They might instead be downstream of the primary pathogenic steps. Given the accumulated evidence, we propose the following model for SCA1 pathogenesis: expansion of the polyglutamine tract alters the conformation of ataxin-1, causing it to misfold. This in turn leads to aberrant protein interactions. Cell specificity is determined by the cell-specific proteins interacting with ataxin-1. Submicroscopic protein aggregation might occur because of protein misfolding, and those aggregates become detectable as NIs as the disease advances. Proteasome redistribution to the NI might contribute to disease progression by disturbing proteolysis and subsequent vital cellular functions.

Keywords: spinocerebellar ataxia type 1; ataxin-1; cerebellum; neurodegeneration; proteasome; chaperones

The dominantly inherited spinocerebellar ataxias (SCAs) are a heterogeneous group of neurological disorders characterized by variable degrees of degeneration of the cerebellum, spinal tracts and brain stem (Greenfield 1954; Koeppen & Barron 1984). Clinical and pathological classification of the SCAs has been very difficult because of intra- and interfamilial variability. The decade of the brain has witnessed an incredible amount of progress in genetic studies of SCAs. So far, the loci for ten ataxias have been mapped and the genes and/or mutations have been identified for eight. The challenge for the next decade is to decipher the functions of the protein products and to unravel disease pathogenesis. The following is a summary of gene function and disease pathogenesis studies in SCA type 1 (SCA1).

SCA1 is characterized by progressive ataxia, dysarthria, amyotrophy and bulbar dysfunction. The typical age of onset is in the third or fourth decade, but early onset in the first decade has been documented in some

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families. Increase in the severity of the phenotype in later generations, a phenomenon known as 'anticipation', has been observed in at least two large SCA1 kindreds (Schut 1950; Zoghbi *et al.* 1988). The disease typically progresses over ten to 15 years, but a more rapidly progressive course has been described in juvenile-onset cases (Zoghbi *et al.* 1988). Pathologically, SCA1 is characterized by the degeneration of cerebellar Purkinje cells, inferior olive neurons and neurons within brainstem cranial nerve nuclei.

The *SCA1* gene was identified in 1993 and the mutation was demonstrated to be an expansion of a translated CAG repeat (Orr *et al.* 1993). Normal alleles contain 6–44 repeats and are always interrupted with one to three CAT nucleotides when the CAG tract contains 21 or more repeats (Chung *et al.* 1993). In contrast, expanded disease alleles contain 39–82 uninterrupted CAG repeats. The *SCA1* gene has a wide pattern of expression in the nervous system and peripheral tissues. The gene product, ataxin-1, is a novel protein that has nuclear localization in neurons and cytoplasmic distribution in peripheral tissues. Within the central nervous system it is abundantly expressed in neurons that are affected by the disease (Purkinje cells and brainstem neurons) and those that are spared (hippocampal and cortical neurons) (Servadio *et al.* 1995).

To gain insight into the normal function of ataxin-1, mice with a targeted deletion in the *Sca1* gene were generated. These mice are viable, fertile and show no evidence of ataxia or neurodegeneration. *Sca1*-null mice do, however, demonstrate decreased exploratory behaviour, pronounced deficits in spatial memory and impaired performance on the rotating-rod apparatus. At the neurophysiological level, studies on area CA1 of the hippocampus revealed decreased paired-pulse facilitation (PPF) but normal long-term potentiation (LTP) and post-tetanic potentiation (PTP) (Matilla *et al.* 1998). These findings prove incontrovertibly that SCA1 is not caused by a loss of function of ataxin-1 and suggest that the protein has some role in learning and memory.

Overexpression of a mutant *SCA1* allele (82 glutamine residues, line BO5) in mice with the use of the Purkinje cell promoter (*Pcp2*) resulted in progressive ataxia and Purkinje cell degeneration (Burright *et al.* 1995). The phenotype was similar when the mutant allele was expressed on a wild-type background or on an *Sca1*-null background, confirming that the SCA1 mutation causes disease via a toxic gain-of-function mechanism. Ataxin-1 aggregates and localizes to a single nuclear inclusion (NI) in the Purkinje cells of SCA1 transgenic mice. This finding prompted the careful evaluation of ataxin-1 distribution in the tissue of SCA1 patients. Ataxin-1 aggregates are detected in brainstem neurons that typically degenerate in this disease (Skinner *et al.* 1997). Intranuclear protein aggregation has been also observed for huntingtin, ataxin-3, ataxin-7, atrophin-1 and the androgen receptor (Davies *et al.* 1997; Hayashi *et al.* 1998; Holmberg *et al.* 1998; Li *et al.* 1998; Paulson *et al.* 1997; Scherzinger *et al.* 1997). An important issue is whether these intranuclear aggregates have a role in initiating pathogenesis or whether they represent a late downstream effect in the pathogenetic process. To address this question, transgenic mice were generated by using a form of mutant ataxin-1 that does not aggregate in transfected cells. This form of ataxin-1 lacks the self-association domain that is necessary for ataxin-1 to interact with itself in yeast two-hybrid studies (Burright *et al.* 1997). Overexpression of ataxin-1 containing 77 CAG repeats but lacking the self-association domain (ataxin-1{77}Δ) in mice with the use of the *Pcp2* promoter resulted in the same phenotype of ataxia and Purkinje cell pathology as in the original BO5 SCA1 transgenic mice. However, the nuclear inclusions were not detected (Klement *et al.* 1998). These results suggest that ataxin-1 aggregates are not necessary to initiate SCA1 pathogenesis.

Immunohistological studies reveal that the SCA1 NIs are ubiquitin-positive and that various components of the proteasome are redistributed to their site, suggesting that the cell's proteolytic machinery is attempting to degrade mutant ataxin-1 (Cummings *et al.* 1998). Even more intriguing is the finding that aggregates of ataxin-1 also stain positively for the molecular chaperone HDJ-2/HSDJ. These results suggest that protein misfolding is responsible for the nuclear aggregates seen in SCA1. This hypothesis is supported by the finding that chaperone overexpression in cell culture subdues ataxin-1 aggregation, perhaps by promoting the recognition of the aberrant polyglutamine repeat protein and allowing its refolding and/or ubiquitin-dependent degradation (Cummings *et al.* 1998).

The issue of selective neuronal degeneration in polyglutamine diseases remains an important aspect of the pathogenesis given that, like ataxin-1, many of the mutant proteins are abundantly expressed in cells spared by the disease. One hypothesis proposes that mutant ataxin-1 might interact with one or more proteins that are more abundantly expressed and/or are essential in the vulnerable neurons. The leucine-rich acidic nuclear protein (LANP) is a candidate for the mediation of SCA1 pathogenesis on the basis of its interactions with mutant ataxin-1 and its patterns of expression and subcellular distribution (Matilla *et al.* 1997). It is interesting to note that LANP interacts with ataxin-1{77}Δ, which is consistent with a role of LANP–ataxin-1 interactions in the early pathogenesis of disease. Present work is focusing on determining the role of LANP in SCA1 pathogenesis with the use of mouse models *in vivo* and on overexpressing the HDJ-2/HSDJ chaperone in mice to assess its role in modulating SCA1 pathogenesis.

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