Molecular Pathogenesis of Spinocerebellar Ataxia Type 1 Disease

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Spinocerebellar ataxia type 1 (SCA1) is an autosomal-dominant neurodegenerative disorder characterized by ataxia and progressive motor deterioration. SCA1 is associated with an elongated polyglutamine tract in ataxin-1, the SCA1 gene product. As summarized in this review, recent studies have clarified the molecular mechanisms of SCA1 pathogenesis and provided direction for future therapeutic approaches. The nucleus is the subcellular site where misfolded mutant ataxin-1 acts to cause SCA1 disease in the cerebellum. The role of these nuclear aggregates is the subject of intensive study. Additional proteins have been identified, whose conformational alterations occurring through interactions with the polyglutamine tract itself or non-polyglutamine regions in ataxin-1 are the cause of SCA-1 cytotoxicity. Therapeutic hope comes from the observations concerning the reduction of nuclear aggregation and alleviation of the pathogenic phenotype by the application of potent inhibitors and RNA interference.

INTRODUCTION

Spinocerebellar ataxia type 1 (SCA1) is an autosomal-dominant neurodegenerative disease that typically has a mid-life onset, and which is characterized by motor symptoms in the absence of cognitive deficits (Orr, Zoghbi, 2000; Zoghbi, 2000; Orr, 2000). SCA1 has the intriguing feature that the disease-causing mutation is the expansion of an unstable trinucleotide repeat, specifically a CAG repeat that encodes the amino acid glutamine in ataxin-1 (Banfi et al., 1994; Orr et al., 1993). Death usually occurs between 10 and 15 years after the onset of symptoms. The clinical features of SCA1 vary depending on the stage of the disease, but typically in addition to ataxia include dysarthria and difficulties in swallowing and breathing. At the pathological level, the most frequent and severe alterations seen in SCA1 patients are the loss of Purkinje cells in the cerebellar cortex and the degeneration of neurons in the inferior olivary nuclei, cerebellar dentate nuclei and red nuclei (Orr and Zoghbi, 2000; Zoghbi, 2000; Orr, 2000). Numerous observations have established that the polyglutamine repeat by itself has a central role in the pathogenesis of polyglutamine diseases, although its effects are strongly modulated by the protein context within which it resides (Michalik and Van Broeckhoven, 2003). The discovery in 1995 of a single, large (approximately 2 nm) nuclear inclusion of mutant ataxin-1 in Purkinje cells of the first transgenic model of SCA1 suggested that polyglutamine toxicity might derive from its ability to form aggregates (Perutz et al., 1994; Thakur and Wetzel, 2002), although nuclear localization, phosphorylation modification and aberrant protein-protein interactions, rather than nuclear aggregation of ataxin-1, appear to be required for initiation of SCA1 pathogenesis both in vivo and in vitro (Chen et al., 2003; Emamian et al., 2003; Hong et al., 2002; Klement et al., 1998; Lim et al., 2008; Matilla et al., 1997; Tsuda et al., 2005) (Fig. 1). Nevertheless, the debate concerning the role of ataxin-1 aggregation and its interaction with other cellular proteins in SCA1 pathogenesis is ongoing. The purpose of this review is to summarize current information on the pathogenesis of SCA1 disease, and to advocate the importance of development of potent inhibitors, gene-based, and stem cell-based therapies for SCA1 disease.

Triplet repeat expansion in polyglutamine diseases
Nine distinct polyglutamine disorders and their involved genes have thus far been identified (Orr and Zoghbi, 2001; Zoghbi, 2000; Orr, 2000; Zoghbi et al., 1991). The nature of the problematic mutation is instability in a triplet-repeat tract (Orr and Zoghbi, 2000; Orr and Zoghbi, 2000). The triplet repeat that is involved in the ataxias consists of a row of three nucleotide bases (cytosine, adenine, guanine) in the disease gene, which encodes the amino acid glutamine (Zoghbi, 2000). In normal genes, CAG may be repeated from 6-35 times, but mutant genes are expanded well beyond their normal length, encompassing 40 to more than 100 triplets (Chung et al., 1993). Because a number of neurodegenerative disorders and their concerned proteins including a number of spinocerebellar ataxias (SCAs; ataxins), Huntington’s disease (HD; huntingtin), dentatorubral-pallidoluysian atrophy (DRPLA; atrophin-1), and spinal and bulbar muscular atrophy (SBMA; androgen receptor), share this expansion of the polyglutamine tracts, they are known collectively as polyglutamine or triplet repeat diseases (Orr and Zoghbi, 2000; Zoghbi, 2000; Orr and Zoghbi, 2000). Interestingly, the longer the expansion is, the more severe the disease is, and the earlier is the onset. The repeat is unstable...
The aggregation displays kinetics of nucleated-growth polymers, biochemical/structural properties of the resulting aggregates and Van Broeckhoven, 2003). The existence of such inclusions indicates the presence of microscopically discernible aggregates of the SCA1 gene product, ataxin-1, in the central nervous system and peripheral tissues, selective degeneration of cerebellar Purkinje cells and brainstem neurons occurs in SCA1 disease (Zoghbi, 1995). Ataxin-1 is found predominantly in the nucleus of neuron cells. Mice that express expanded ataxin-1 (82Q) with mutated nuclear localization sequence (NLS), ataxin-1K772T, never develop Purkinje cell pathology or motor dysfunction (Klement et al., 1998). In the transgenic mouse, ataxin-1 is diffusely distributed throughout the cytoplasm and does not form aggregates, even in one-year-old mice. Therefore, nuclear localization of the expanded ataxin-1 is clearly critical for the pathogenesis of SCA1 disease. A pathological feature of most of polyglutamine disorders is the presence of microscopically discernible aggregates of the mutant proteins in the nucleus and cytoplasm of affected neurons (Becher et al., 1998; DiFiglia et al., 1997; Holmberg et al., 1998; Huynh et al., 1999; Li et al., 1998; Paulson et al., 1997; Skinner et al., 1997). The existence of such inclusions indicates that proteins containing an expanded polyglutamine tract acquire a tendency to aggregate, which could be crucial to the pathogenesis of these diseases (Chen et al., 2003; Michailik and Van Broeckhoven, 2003). In vitro studies of polyglutamine aggregation have defined both the aggregation kinetics and the biochemical/structural properties of the resulting aggregates (Chen et al., 2001; Perutz et al., 1994; Scherzinger et al., 1997). The aggregation displays kinetics of nucleated-growth polymerization, with a prolonged lag-phase required to form an aggregate, and a fast extension phase for joining the polyglutamine monomer to the growing aggregate (Chen et al., 2001; Scherzinger et al., 1999).

Furthermore, the aggregation propensity of ataxin-1 could be derived from its ability to self-interact (Burright et al., 1997). To reveal the molecular details of this interaction, Burright et al. (1997) identified the multimerization region and self-association region (SAR) using yeast two-hybrid system, and another study characterized a dimerization domain (AXH) for protein-protein interaction (de Chiara et al., 2003) or RNA binding motif in ataxin-1 (Yue et al., 2001). Also, the polar zipper model proposes that polyglutamine tracts forming extended anti-parallel beta-strands are held together by an extensive network of hydrogen bonds between both the main-chain and side-chain amides. However, the precise role of polyglutamine molecules in the aggregation of ataxin-1 is still unknown and remains a subject of intense scrutiny.

**Role of ataxin-1 aggregates in SCA1 disease**

A pathological hallmark of polyglutamine diseases as well as SCA1 is the presence of the small and large aggregates containing the mutant polyglutamine proteins (Davies et al., 1997; Sanchez et al., 2003; Skinner et al., 1997; Yang et al., 2002). In case of SCA1 transgenic mice and patients containing mutant ataxin-1 (82Q), the aggregates are positive for ubiquitin (Cummings et al., 1998) and the components of proteasome and the HDJ-2/HSDJ chaperone protein (Cummings et al., 1998) that are involved in quality control of protein surveillance machinery (Schmidt et al., 2002). Since the moment of their identification, the aggregates were viewed as having a central role in polyglutamine-mediated pathogenesis (Taroni and DiDonato, 2004; Zoghbi and Orr, 2009). However, the primary pathogenic role of the aggregates is still unclear (Sisodia, 1998; Zoghbi and Orr, 2009).

Although efforts to uncover the molecular mechanisms of polyglutamine aggregation are ongoing, one must inquire whether aggregates constitute the sole mechanism in the cascade of events leading to neuronal dysfunction. Several influential studies argued that the aggregates might not be the main mechanism of polyglutamine toxicity. SCA1 knock-in mice that introduced an expanded repeat of 154 CAGs into the endogenous mouse Sca1 locus (Sca1154C) showed degeneration of cerebellar Purkinje cells, although these neurons did not develop prominent aggregates (Watase et al., 2002). One study showed a comparable ataxic phenotype in two distinct SCA1 transgenic models (Klement et al., 1998); one expressing the full-length mutant ataxin-1 (82Q) developed ataxia with ataxin-1 aggregates within the nuclei of cerebellar Purkinje cells, while the other carrying an internal deletion within SARs developed ataxia and Purkinje cell pathology. In addition, a subsequent analysis revealed that ataxia in the transgenic mice lacking aggregates within a Purkinje cell was non-progressive (Skinner et al., 2002). These studies suggest that nuclear aggregation of ataxin-1 appears not to be required for initiation of cerebellar Purkinje cell pathogenesis of transgenic mice, although nuclear localization of ataxin-1 is necessary. It is important to note that the deletion of 122 amino acids might impair ataxin-1 in various ways that include folding, turnover rate, or ability to interact with other cellular proteins (Orr and Zoghbi, 2001). However, immunohistochemical studies of brain tissues obtained from SCA1 patients and transgenic mice as well as cell cultures indicate that the aggregates contain chaperones, and ubiquitin and proteasome subunits of the ubiquitin proteasomal pathway (Cummings et al., 1998; 1999a; 1999b; 2001). In one of these studies, ataxin-1 (82Q) transgenic mice were crossed with mice lacking expres-