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ScienceDirect
Experimental Neurology 208 (2007) 207–215
Experimental Neurology
www.elsevier.com/locate/yexnr

Ataxin-2 mediated cell death is dependent on domains downstream of the polyQ repeat

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Received 17 April 2007; revised 4 July 2007; accepted 21 July 2007
Available online 28 August 2007

Abstract

Spinocerebellar ataxia 2 (SCA2) belongs to the group of neurodegenerative diseases caused by expansion of a polyglutamine (polyQ) domain. Overexpression of mutant ataxin-2 causes cell death and Golgi dispersion in cell culture as well as morphologic and functional changes in mouse models. To further define the mechanism of ataxin-2 induced cell death, we compared the cytotoxic effects of different domains of normal and mutant ataxin-2. N-terminal truncated ataxin-2^{N} with expanded polyQ repeats did not form intranuclear inclusion and was less cytotoxic than the corresponding full-length ataxin-2. Ataxin-2^{del42}[Q22], which lacks 42 amino acids (aa) within the Lsm-associated domain (LsmAD) necessary for Golgi localization, showed a diffuse cytoplasmic localization and was more toxic than wild type ataxin-2[Q22]. Mutant ataxin-2^{del42}[Q108] displayed the same toxicity as ataxin-2[Q108], but did not disperse the Golgi apparatus to the extent seen with full-length mutant proteins. These observations confirm that ataxin-2 cytotoxicity increases with increasing polyQ expansion and Golgi dispersion and indicate that, in contrast to other polyQ diseases, N-terminal fragments containing the polyQ repeat are less toxic than full-length ataxin-2. Deletion of 42 aa in the Lsm-AD in ataxin-2 results in cytotoxicity without significant abnormalities in the Golgi apparatus. These findings suggest that the C-terminal domains are important for ataxin-2 cytotoxicity and that Golgi abnormalities may not be primary in the pathogenic process.

Keywords: Spinocerebellar ataxia type 2; SCA2; PolyQ protein; Neurodegenerative disease; Ls-associated domain; Golgi dispersion; Cytotoxicity; COS1; Overexpression; Cell model

Introduction

Spinocerebellar ataxia 2 (SCA2) is a polyglutamine (polyQ) neurodegenerative disease that results from the expansion of a CAG trinucleotide repeat in the coding region of the ataxin-2 gene (Imbert et al., 1996; Pulst et al., 1996; Sanpei et al., 1996). The normal SCA2 gene sequence contains 14–31 CAG repeats, and symptoms of SCA2 occur when the CAG repeats are increased to 32 or greater. Increasing the length of the polyQ repeat is associated with more severe clinical symptoms and an earlier onset (Estrada et al., 1999; Imbert et al., 1996; Pulst et al., 1996, 2005; Sanpei, 1999). Mice expressing mutant ataxin-2 with 58 glutamines (ataxin-2[Q58]) showed progressive functional deficits accompanied by loss of the Purkinje cell dendritic arbor and finally loss of Purkinje cells (Huynh et al., 2000).

Ataxin-2 is a basic protein except for the acidic domain (amino acids 280–481) downstream of the polyQ repeat (Shibata et al., 2000). Within the acidic domain are putative caspase-3 motif (aa396–399), RNA splicing motifs (Lsm; like sm; aa254–345) and an Lsm-associated domain (LsmAD, aa353–475). The Lsm/LsmAD contains a putative clathrin-mediated trans-Golgi signal (aa414–416) and an endoplasmic reticulum (ER) exit signal (aa426–428). In addition, ataxin-2

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doi:10.1016/j.expneurol.2007.07.023

also contains a PABP/Pab1 binding motif (PAM2) downstream of the LsmAD (aa908–925). The yeast homolog of ataxin-2, Pbp1, which also contains the Lsm domain similar to ataxin-2, regulates polyadenylation and binds to the yeast poly(A) binding protein, Pabp1 (Mangus et al., 1998, 2003). Pabp1 is an ortholog of the human poly(A)-binding protein 1 (PABP1) which also binds to human ataxin-2 (Ralser et al., 2005). The presence of the Lsm domain in both human and yeast ataxin-2 suggests that ataxin-2 can bind to RNA and regulate RNA metabolism and/or RNA processing in common with other Lsm domain proteins (Albrecht et al., 2004). The identification of A2BP1 (ataxin-2 binding protein 1) as an interactor of ataxin-2 by yeast two-hybrid assays (Shibata et al., 2000) further supports the notion that ataxin-2 is involved in RNA regulation. A2BP1 also contains RNA recognition domains. The *C. elegans* ortholog of A2BP1, Fox-1, interacts with RNA and controls tissue specific alternative splicing (Jin et al., 2003).

In polyQ diseases as well as in other neurodegenerative diseases such as Parkinson and Alzheimer disease, neuronal death is associated with cytoplasmic or intranuclear aggregation or accumulation of mutant proteins (DiFiglia et al., 1997; Huynh et al., 1999, 2000; Klement et al., 1998; Koyano et al., 2002, 2000; Paulson et al., 1997; Uchihara et al., 2001). Although intranuclear aggregates are seen in a small number of brain stem neurons in SCA2 patients (Koyano et al., 2002, 2000) and COS1 cells transiently transfected with ataxin-2[Q108] (Huynh et al., 2003), cytoplasmic aggregates predominate without the formation of large inclusion bodies, and no nuclear inclusions have been observed in Purkinje neurons of SCA2 patients (Huynh et al., 1999, 2000; Koyano et al., 2002). Co-expression with parkin, an E3 ubiquitin ligase, alleviated the cytotoxicity of mutant ataxin-2 (Huynh et al., 2007).

Wild type ataxin-2[Q22] colocalized with a trans-Golgi 58K marker and was found in the Golgi/endosomal fraction after differential centrifugation (Huynh et al., 1999, 2003; Shibata et al., 2000). Deletion of a 42 amino acid (aa) domain (ataxin-2^{del42}[Q22]) that resides within the LsmAD resulted in loss of Golgi association (Huynh et al., 2003). Ataxin-2 with an expanded polyQ repeat disrupted the normal morphology of the Golgi complex and increased cell death.

We now report the subcellular distribution, Golgi morphology and viability of COS cells transfected with different ataxin-2 domains. These experiments were carried out with proteins containing the normal 22 glutamine repeat domain and proteins with the polyQ domain expanded to 58 and 108Q.

Materials and methods

Plasmid construction

The pEGFPC2-ataxin-2[Q22], pEGFPC2-ataxin-2[Q58] and pGFP-ataxin-2[Q108] plasmids were constructed as previously described (Huynh et al., 2003) and confirmed by sequencing. To construct the pEGFPC2-ataxin-2^{N}[Q22] plasmid, the pEGFP-SCA2[Q22] vector containing the full-length ataxin-2 was digested with *Bam*HI, which cuts at nucleotide 1349. This plasmid was designated pEGFPC2-ataxin-2[Q22]^{N}. The ataxin-2^{del42}[Q22] plasmid was constructed by digesting the plasmid pGFPC2-SCA2 [Q22] with *Sna*BI and *Xho*I at nucleotides 1404 and 1530, respectively. This restriction digest removed amino acid residues 414 to 456 within the LsmAD. The deleted amino acid sequence contains a putative clathrin-mediated trans-Golgi signal (aa residues 414–416) and a putative ER exit signal (aa residues 426–428) (Figueroa and Pulst, 2003; Pulst et al., 1996). The *Xho*I 5′-overhang was blunt-ended by T4 DNA polymerase and was religated in-frame with the *Sna*BI-digested blunt-end by T4 DNA ligase. This plasmid construct was designated pEGFPC2-ataxin-2^{del42}[Q22]. This plasmid was inadvertently described as carrying a 43 aa deletion instead of a 42 aa deletion by Huynh et al. (2003). Plasmids pEGFC2-ataxin-2^{del42}[Q58] and pEGFPC2-ataxin-2^{del42}[Q108] were generated by transferring the 3′-terminal *Bam*HI restricted fragment of pEGFPC2-ataxin-2^{del42}[Q22] to the 5′-*Bam*HI restricted fragment of either pEGFC-ataxin-2[Q58] or pEGFPC2-ataxin-2[Q108].

Sequence analysis of all ataxin-2 constructs confirmed the presence of the respective CAG repeats and the sites of N-terminal truncation or interstitial deletion. Sequence analysis of the C-terminus identified presence of an additional 6 nucleotides encoding Gly-Lys not reported in the original SCA2 cDNA sequence. We have subsequently found that they are due to use of an alternative splice site in the intron between exons 18 and 19. This cDNA splice variant is abundant in human and mouse brain and was originally reported in the mouse cDNA sequence (Nechiporuk et al., 1998). It has been reported for several other species as well.

Cell culture

All culture media (DMEM, FBS and penicillin/streptomycin) were purchased from Invitrogen Inc. COS1 cells were grown in DMEM medium supplemented with 10% FBS and penicillin/streptomycin in an incubator at 37 °C and 5% CO_2. Cells were seeded at a subcultivation ratio of 1:3 to 1:6 every 3 days. One day prior to transfection, 200,000 cells were seeded on poly-lysine coated 1 cm^2 coverslip placed in 6-well dishes.

Transient transfection

Plasmid DNA was mixed with polyfect transfection reagent and transiently transfected into overnight COS1 cell cultures according to the manufacturer's protocol (Qiagen). Twenty-four hours after transfection, cells were collected for either cell death assay or immunofluorescent staining. To achieve equal transfection efficiencies for all plasmids, we measured DNA concentrations by spectrophotometry and independent confirmation by serial dilutions of ethidium-bromide-stained agarose gels. Equal molar ratios of the plasmids were used for each experiment.

Trypan blue exclusion assay

We used the trypan blue exclusion assay to determine cell death. Twenty-four hours after transfection, cells were incubated with an equal volume of 0.4% trypan blue for 5 min. Labels were covered to blind the individual (SN) scoring the assay. Trypan