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Minireview

Molecules and Cells

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, 2000; Zoghbi and 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, 2001; Zoghbi and 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 um) 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., 1997b; 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 and Orr, 2000; 2009; Zoghbi et al., 1991). The nature of the problematic mutation is instability in a triplet-repeat tract (Orr and Zoghbi, 2007; Zoghbi and Orr, 2000; 2009). 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), dentatorubralpallidoluysian atrophy (DRPLA; atrophin-1), and spinal and bulbar muscular atrophy (SBMA; androgen receptor), share this expansion of the polyglutamine tract, they are known collectively as polyglutamine or triplet repeat diseases (Orr and Zoghbi, 2007; Zoghbi, 2000; Zoghbi and Orr, 2000; 2009). Interestingly, the longer the expansion is, the more severe the disease is, and the earlier is the onset. The repeat is unstable

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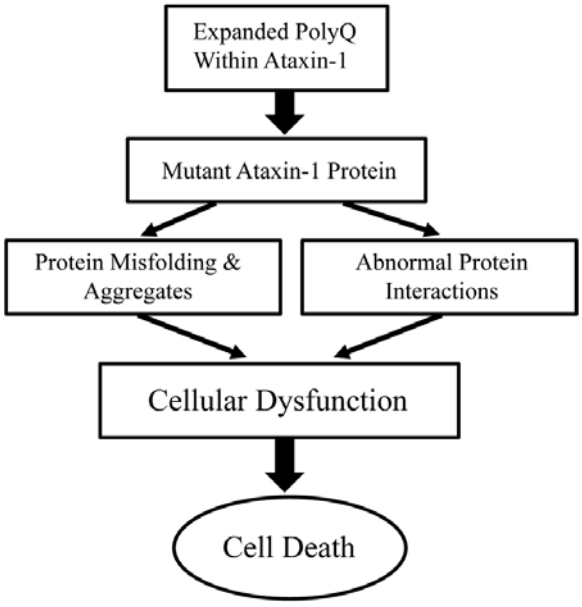

Fig. 1. A schematic flow showing the progress of cellular dysfunction in SCA1.

and expands as it is passed from one generation to the next, particularly if the affected parent is the male, explaining the phenomenon of anticipation (Zoghbi and Orr, 1995). The expansion of triple repeat tracts could be caused by errors of the replication machinery (Kang et al., 1995).

Expression and aggregation of ataxin-1 in the nucleus

In the cerebellum, the SCA1 gene product, ataxin-1, is expressed in various types of neurons, but immunofluorescence analysis has indicated that its' expression in granule neurons is much lower than that in Purkinje neurons (Watase et al., 2002). The ataxin-1 protein is found also in peripheral tissues (Servadio et al., 1995). Despite the broad expression patterns of 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-1^{K772T}, 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; Michalik and Van Broeckhoven, 2003). *In vitro* studies of polyglutamine aggregation has 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 aggregation, 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 patholgical 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 (*Sca1*^{154Q}) 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-

sion of *Ube3a* gene, which encodes the E6-AP (E6-associated protein). The absence of E6-AP (also known as E3 ubiqutin ligase) delayed the appearance of nuclear aggregates but accelerated both the onset of ataxic phenotype and the morphological deterioration of cerebellar Purkinje cells in double transgenic mice deficient in E6-AP (Cummings et al., 1999b). These data suggest that the ataxin-1 aggregates can trigger a stress response to diminish their burden in a Purkinje cell that plays a critical role in toxicity. The SCA1 transgenic mice studies provide compelling evidence that ataxin-1 aggregates might not constitute the only toxic species rather than completely deny their involvement for the pathogenesis of SCA1 disease.

Cellular dysfunction through abnormal interactions in SCA1 disease

Another strategy to assess possible biological and pathological functions of ataxin-1 in SCA1 disease is to identify ataxin-1-interacting proteins in yeast two-hybrid systems and cell culture systems using affinity purification and immunoprecipitation methods. The nuclear protein LANP is predominantly expressed in the nuclei of cerebellar Purkinje cells and has a stronger interaction with mutant ataxin-1 containing expanded polyglutamine tract than does wild-type ataxin-1 (Matilla et al., 1997). Ubiquitin-specific protein USP7, while predominantly expressed in the nucleus, is also present in a minimum of the nuclear bodies associated with the nuclear matrix and has a weaker interaction with mutant ataxin-1 than does wild-type ataxin-1 (Hong et al., 2002). To date, other ataxin-1-interacting proteins that are regulated by the length of the polyglutamine tract have been identified including polyglutamine binding protein 1 (PQBP1), 14-3-3, zinc-finger transcription factor Sp1, transcriptional repressor Capicua, and RNA-binding motif protein 17 (RBM17) (Chen et al., 2003; Goold et al., 2007; Lam et al., 2006; Lim et al., 2008; Okazawa et al., 2002). These data suggest that the expanded polyglutamine could change the conformation of ataxin-1 protein and trigger a series of aberrant interactions with cellular proteins. Interestingly, most of the proteins interact with ataxin-1 through the C-terminal region containing the AXH domain. Recently, it was reported that growth factor independent-1 (Gfi-1) interacts with the AXH domain, with the interaction being necessary to generate the SCA1 phenotype, but not being influenced by the length of polyglutamine tract (Tsuda et al., 2005). These results suggest that the expanded polyglutamine may alter biological functions of Gfi-1 protein itself or other Gfi-1-interacting proteins.

Among the known ataxin-1-interacting proteins (Table 1), most are involved in the regulation of transcription (Cvetanovic et al., 2007; Goold et al., 2007; Lam et al., 2006; Lee et al., 2008; Mizutani et al., 2005; Serra et al., 2006; Tsuda et al., 2005), RNA processing (Hong et al., 2003; Irwin et al., 2005; Lim et al., 2006; 2008; Okazawa et al., 2002), protein modifications (Al-Ramahi et al., 2006; Chen et al., 2003; Emamian et al., 2003; Hong et al., 2008; Riley et al., 2005; Ueda et al., 2002), stabilization (Chen et al., 2003; Hong et al., 2002), and signal transduction (Gatchel et al., 2008; Goold et al., 2007; Serra et al., 2004). Ser776 has been identified as the major phosphorylation site of ataxin-1 in the nucleus of cerebellar Purkinje cells (Emamian et al., 2003), a site of neurotoxicity. This phosphorylation might modulate ataxin-1 aggregation since expanded ataxin-1 containing an alanine in place of serine (Ala776) fails to form aggregates in cultured cells, and transgenic mice expressing expanded ataxin-1 (Ala776) in cerebellar Purkinje cells fail to develop ataxia and show considerable reduction in inclusion formation and Purkinje cell degeneration.

Specific isoforms of the 14-3-3 protein interact with ataxin-1, in a reaction that requires phophorylation of the Ser776 residue of ataxin-1 (Chen et al., 2003). One of many functions of 14-3-3 is to bind to and stabilize phosphorylated substrates, consistent with the suggestion that 14-3-3 stabilizes expanded and phosphorylated ataxin-1, and increases cellular levels of abnormally folded ataxin-1, which in turn promotes neurodegeneration of SCA1 disease in the cerebellum (Chen et al., 2003; Paulson, 2003). These studies suggest that expanded polyglutamine and nuclear localization do not cause ataxin-1 neurotoxicity, but protein modification involving Ser776 may contribute to SCA1 pathogenesis, and that the ataxin-1 phosphorylation and 14-3-3 protein interaction cooperate to modulate neurotoxicity of expanded ataxin-1.

In addition, transcriptional dysregulation mediated by expanded ataxin-1 is an important feature in the pathogenesis of SCA1 disease. Several groups have reported that perturbed interaction between expanded ataxin-1 and transcription factors such as LANP, PQBP1, Gfi-1, SMRT, Boat, and Sp1 might exert the deleterious effects of expanded ataxin-1 (Goold et al., 2007; Matilla et al., 1997a; Mizutani et al., 2005; Okuda et al., 2003; Tsai et al., 2004; Tsuda et al., 2005).

Furthermore, recent reports independently demonstrated that the molecular pathways most likely implicated in SCA1 disease include dopamine receptor D2 (Drd2), retinoic acid/thyroid hormone, Wnt-signaling, and insulin-like growth factor (IGF) pathways (Gatchel et al., 2008; Goold et al., 2007).

However, although the expanded polyglutamine in ataxin-1 can be toxic to cells, it seems not to be sufficient to cause SCA1 disease. A recent study found that the expanded polyglutamine in ataxin-1 protein shifts the balance of cellular protein complex as a novel mechanism of SCA1 pathogenesis (Lim et al., 2008). The researchers identified a protein called RBM17 that interacts only with phosphorylated ataxin-1, and showed that the interaction was strongly enhanced by an expanded polyglutamine tract. Interestingly, they found that there are two distinct and large native complexes containing ataxin-1: one containing RBM17 and the other containing capicua (CIC). They also found that the expanded polyglutamine tract alters the proportion of mutant ataxin-1 to form specific protein complexes *in vivo*, and that the components of these complexes compete for interaction with ataxin-1. The expanded ataxin-1 preferentially formed a complex with RBM17, which may cause SCA1 disease by a gain-of-function mechanism, whereas wild-type ataxin-1 was present in the CIC-containing complex that caused a loss-of-function mechanism (Lam et al., 2006; Lim et al., 2008). These observations suggest that, when ataxin-1 is mutated, a simultaneous increase in the levels of RBM17-ataxin-1 complexes and decrease in those of CIC-ataxin-1 complexes may disrupt the critical balance essential for proper cellular function. However, the clear mechanism by which the expanded polyglutamine tract causes both toxic gain-of-function and loss-of-function of the disease-causing protein has not yet been clarified.

Potential therapies for SCA1 disease

Recently, potential therapies for SCA1 have been intensively studied using animal models and molecular chaperones. Increased levels of certain molecular chaperones such as heat-shock protein 70 (HSP70) and HSP40 suppress both aggregation and toxicity of mutant ataxin-1 in cell culture systems (Cummings et al., 1998), and in *Drosophila* and mouse models of HD and SCA1 disease (Chan et al., 2000; Fernandez-Funez et al., 2000; Kazemi-Esfarjani and Benzer, 2000; Muchowski et al., 2000). Besides molecular chaperones, other polyglutamine-mediated aggregation-suppressing agents such as chemical

Table 1. Ataxin-1-interacting proteins and signaling pathways

Interacting proteins and signalings	Cellular pathways	Interaction regions within ataxin-1	Poly Q modulation
PQBP1	Transcription	Not shown	Yes
Gfi-1/senseless		AXH	No
Tip60		A region including SAR	Yes
Boat		SAR	Yes
UbcH6		C-Terminus	No
LANP		C-Terminus	Yes
Capicua		AXH	No
SMART		Not shown	No
Sp1		AXH	Yes
A1Up	RNA processing	SAR	No
PQBP1		Not shown	Yes
p80 coilin		C-Terminus	No
TAP/NXF1		Not shown	Unknown
RBM9		Not shown	Unknown
PUM1		Not shown	Unknown
RBM17		C-Terminus	Yes
Ubiquitin	Ubiquitination	Not shown	Yes
CHIP		Not shown	No
UbcH6		C-Terminus	No
Phophate	Phosphorylation	C-Terminus	Yes
Akt		Not shown	Unknown
Sumo-1	Sumoylation	Multiple domains	Yes
USP7	Stabilization	C-Terminus	Yes
14-3-3		C-Terminus	Yes
Glutamate-signaling	Signal transduction		
Drd2-signaling			
RA/thyroid hormone-signaling			
Wnt-signaling			
IGF			

chaperones (Yoshida et al., 2002), synthetic or recombinant peptides (Nagai et al., 2000; Kazantsev et al., 2002; Ren et al., 2001), intracellular antibodies (Khoshnan et al., 2002; Lecerf et al., 2001), various disaccharides (Tanaka et al., 2004), and certain chemicals (Heiser et al., 2000; 2002; Sanchez et al., 2003) also attenuate polyglutamine toxicity. Alternatively, histone deacetylase inhibitor (HDACi) suppresses polyglutamine toxicity in cell and *Drosophila* models (McCampbell et al., 2001; Steffan et al., 2001). An ideal treatment for polyglutamine diseases would be a combination of these and other therapeutic strategies. Compounds that inhibit the PI-3K/Akt pathway or small peptides that prevent 14-3-3/ataxin-1 interactions might play a role in SCA1 treatment (Paulson, 2003). Furthermore, the androgen-blockage drug leuprorelin rescues the polyglutamine-dependent phenotype in a transgenic mouse model of SBMA (Katsuno et al., 2003). However, there is no effective therapy due to lack of target specificity and low effectivity for SCA1 or other neurodegenerative diseases caused by the expanded polyglutamine tract.

Does inhibition of mutant ataxin-1 expression and/or reduction of a particular mutant ataxin-1 complex containing RBM17 improve behavior and neuropathology of SCA1? More recently, gene silencing of the mutant allele through RNA interference has proved useful method to prevent target gene expression both *in vitro* (cell culture) and *in vivo* (brain) (Xia et al., 2004). Introduction of recombinant adeno-associated virus vectors expressing short hairpin RNAs directed against the human mutant ataxin-1 gene improve the behavioral phenotype in SCA1 animal models (Xia et al., 2004). In SCA1 patients, however, short hairpin RNAs would problematically target both the mutant and wild-type allele, although ataxin-1 knock-out mice do not display SCA1 phenotype.

Polyglutamine disorders seem to be relatively simple models of neurodegeneration, but there is currently no cure available because their cellular and pathophysiological mechanisms are complex. Disease modifying therapies such as RNA interference and pharmacological intervention target the disease genes or pathogenic pathways, while cell-based therapies attempt to replace dysfunctional or dying and/or dead cells (Fig. 2).

Stem cell therapy in the patients with neurodegenerative diseases, such as Parkinson's disease, HD, and spinal cord injury, is garnering increased attention for its potential to cure patho-

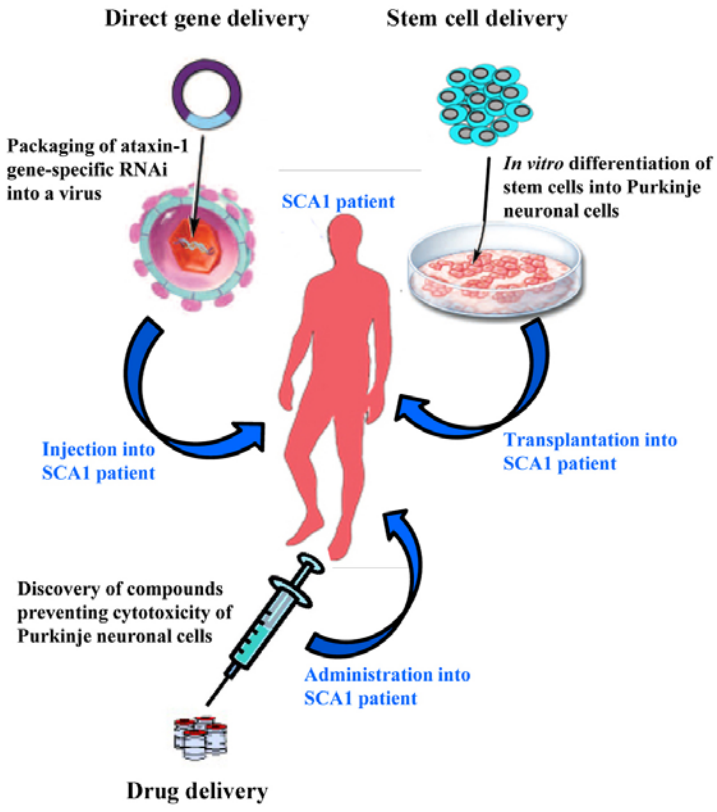

Fig. 2. Drawing illustrating potential therapies in SCA1. Delivery of therapeutic genes and/or compounds and/or stem cells into the SCA1 patient is represented by blue large curved arrows. SCA1, Spinocerebellar Ataxia Type 1; RNAi, RNA interference.

logical symptoms by replacing lost neuronal cells. A recent study reported the induction of precursor cells of cerebellar neurons such as Purkinje cells and granule cells, from embryonic stem (ES) cells using an efficient differentiation method with BMP4 and FGF8 signaling molecules (Su et al., 2006). Interestingly, the researchers generated 1-3% Purkinje cell-like neurons out of total postmitotic neurons from the ES cells. An intriguing challenge is to investigate function of ES cell-derived Purkinje cells in SCA1 animal models. In addition, generation of Purkinje cells from ES cells should be useful for *in vitro* study on pathogenesis and drug development for SCA1 and other diseases. However, many factors that are involved in the differentiation, survival, and maturation of stem cells in a host brain should be completely understood for the clinical application of stem cell therapy in the future.

CONCLUSIONS

In this review, we have concisely summarized our current understanding of some aspects of the pathogenesis of SCA1 disease: localization and aggregation of misfolded ataxin-1 protein, a number of ataxin-1-interacting proteins, and potential factors to alleviate SCA1 disease. For the disease to develop, the mutant ataxin-1 protein containing the expanded-polyglutamine tract should enter the nucleus of a susceptible neuron. In the nucleus, other cellular proteins interact with the polyglutamine tract itself or another protein context of ataxin-1, and can cause an alteration in various cellular processes or pathways. The formation of aggregates by ataxin-1 does not constitute the only toxic mechanism in pathogenesis of SCA1 disease. Intensive ongoing studies are providing a better understanding of molecular and pathological mechanisms of SCA1 disease, and these data are leading to new therapeutic strategies to cure patients with SCA1 and other neurodegenerative diseases.

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