Seipinopathy: a novel endoplasmic reticulum stress-associated disease

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The Seipin/BSCL2 gene was originally identified as a loss-of-function gene for congenital generalized lipodystrophy type 2 (CGL2), a condition characterized by severe lipoatrophy, insulin resistance, hypertriglyceridaemia and mental retardation. Recently, gain-of-toxic-function mutations (namely, mutations N88S and S90L) in the seipin gene have been identified in autosomal dominant motor neuron diseases such as Silver syndrome/spastic paraplegia 17 (SPG17) (OMIM #270685) and distal hereditary motor neuropathy type V (dHMN-V) (OMIM #182960). Detailed phenotypic analyses have revealed that upper motor neurons, lower motor neurons and peripheral motor axons are variously affected in patients with these mutations. The clinical spectrum for these mutations is broad, encompassing Silver syndrome, some variants of Charcot-Marie-Tooth disease type 2, dHMN-V and spastic paraplegia, even within a common pedigree. Therefore, we propose that seipin-related motor neuron diseases can be collectively referred to as ‘seipinopathies’. Expression of the seipin protein can be detected in motor neurons in the spinal cord and white matter in the frontal lobe. This is consistent with the distribution of seipinopathies in the upper and lower motor neurons. Recent studies have shown that seipin, an endoplasmic reticulum (ER)-resident membrane protein, is an N-glycosylated protein that is proteolytically cleaved into N- and C-terminal fragments and is polyubiquitinated. Interestingly, the N88S and S90L mutations are in the N-glycosylation motif, and these mutations enhance ubiquitination and degradation of seipin by the ubiquitin–proteasome system (UPS). Furthermore, both mutations appear to result in proteins that are improperly folded, which leads to accumulation of the mutant protein in the ER. We have shown that expression of mutant forms of seipin in cultured cells activates the unfolded protein response (UPR) pathway and induces ER stress-mediated cell death. These findings suggest that seipinopathies are novel conformational diseases and that neurodegeneration in these diseases is tightly associated with ER stress, which has recently been reported to be associated with other neurodegenerative diseases. Further study of the pathological mechanisms of the mutant forms of seipin may lead to important new insights into motor neuron diseases, including other spastic paraplegia diseases and amyotrophic lateral sclerosis.

Keywords: BSCL2; endoplasmic reticulum stress; lipodystrophy; motor neuron disease; seipin; unfolded protein response

Abbreviations: AGPAT2 = 1-acyl-sn-glycerol-3-phosphate acyltransferase beta; ALS = amyotrophic lateral sclerosis; BSCL2 = Berardinelli–Seip congenital lipodystrophy type 2; CGL2 = congenital generalized lipodystrophy type 2; CMT = Charcot-Marie-Tooth disease; dHMN-V = distal hereditary motor neuropathy type V; ER = endoplasmic reticulum; GARS = glycyl-tRNA synthetase; HSP = hereditary spastic paraplegia; SPG17 = spastic paraplegia 17; UPR = unfolded protein response
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Introduction

Growing evidence indicates that various neurodegenerative diseases such as Alzheimer’s disease, polyglutamine diseases and Parkinson’s disease may have a common pathological feature, namely, protein misfolding, aggregation and accumulation, in affected brain regions (DiFiglia et al., 1997; Tran and Miller, 1999; Kopito, 2000; Kopito and Ron, 2000; Zhang and Kaufman, 2006). It is therefore thought that dysfunction in the protein quality control system plays a significant role in these types of neurodegeneration. An intracellular organelle, the endoplasmic reticulum (ER), is the cell’s quality control site for ensuring accurate folding of secretory and membrane proteins. Pathological and biochemical stimuli that perturb ER function can induce ER stress, a condition characterized by accumulation of misfolded proteins within the ER. Environmental stimuli that induce ER stress include glucose starvation and oxygen stress; any physiological or genetic factors that disrupt glycosylation, calcium homeostasis and correct protein folding or sorting will also contribute to ER stress.

A highly conserved intracellular signalling pathway known as the unfolded protein response (UPR) is activated in response to ER stress (Kaufman, 1999; Liu and Kaufman, 2003; Shen et al., 2004; Hetz et al., 2006; Ron and Walter, 2007; Rutkowski and Kaufman, 2007). UPR first enables the cell to reduce the unfolded protein load in the ER by attenuating translation of new protein and up-regulating protein folding and degradation pathways. However, if ER stress cannot be relieved, the UPR initiates proapoptotic pathways. Cells subjected to ER stress up-regulate expression of the pro-apoptotic transcription factor CHOP, stimulate the pro-apoptotic JNK signalling pathway and activate caspase-12 (Nakagawa et al., 2000; Urano et al., 2000; Nishitoh et al., 2002). Thus, apoptosis ensues if the burden of accumulated misfolded protein cannot be relieved by the UPR. Recently, disruption of ER homeostasis and up-regulation of the UPR has been observed in various neurological diseases, including Alzheimer’s disease, Parkinson’s disease, amyotrophic lateral sclerosis (ALS), prion disease, expanded polyglutamine tract diseases, Charcot-Marie-Tooth disease (CMT) and stroke (Katayama et al., 1999; Nakagawa et al., 2000; Imai et al., 2000; Ito et al., 2001, 2004; Nishitoh et al., 2002; Hetz et al., 2003; Tobisawa et al., 2003; Kikuchi et al., 2006; Lindholm et al., 2006; Turner and Atkin, 2006; Zhang and Kaufman, 2006; Pennuto et al., 2008). Understanding the UPR and the protein quality control mechanism in the ER is therefore necessary to elucidate the molecular basis of these neurodegenerative diseases and develop novel therapies based on these findings (Burrows et al., 2000; Bonapace et al., 2004; Boyce et al., 2005; Kubota et al., 2006; de Almeida et al., 2007; Takizawa et al., 2007). However, neurodegenerative pathways are diverse, and whether ER stress can play a major role in the mechanism of neurodegeneration remains controversial.

Recently, we revealed that a novel motor neuropathy, seipin/Berardinelli-Seip congenital lipodystrophy type 2 (BSCL2)-related motor neuron disease, is tightly associated with ER stress in a series of in vivo studies. Seipin, an ER-resident glycoprotein, has been implicated in several distinct hereditary diseases: recessive congenital generalized lipodystrophy (CGL) (Berardinelli, 1954; Seip, 1959; Seip and Trygstad, 1996) and the dominant motor neuron diseases Silver syndrome and distal hereditary motor neuropathy type V (dHMN-V) (Magre et al., 2001; Agarwal and Garg, 2004; Windpassinger et al., 2004). It is interesting that upper motor neurons, lower motor neurons and peripheral motor axons are affected differently in the various seipin-related motor neuron diseases (Irobi et al., 2004; Auer-Grimbach et al., 2005). Therefore, we propose the novel term ‘seipinopathy’ to refer to such diseases (Ito and Suzuki, 2007b; Ito et al., 2008).

Our studies demonstrated that seipinopathies are novel protein conformational diseases in which mutant proteins undergo a conformational rearrangement that leads to aggregation. The molecular basis underlying these diseases may be intimately linked to ER stress response pathways (Ito and Suzuki, 2007a, 2007b). Mutations N88S and S90L in the N-glycosylation site of seipin, which is associated with the motor neuron disease state, cause accumulation of unfolded protein in the ER, leading to UPR and cell death. We consider ER stress to be a critical step in the neurodegeneration associated with seipinopathy, and understanding of this pathogenesis should lead to important new insights into protein conformational disease, especially ER stress-related neurological diseases. This review offers an overview of the clinical features of seipin-related diseases and the biochemical characteristics of the seipin protein. Thereafter, we describe evidence of ER dysfunction in seipinopathy and discuss possible new therapeutic strategies against ER stress-related diseases.

Identification of the mutation in the seipin/BSCL2 gene associated with Silver syndrome and dHMN-V

Silver syndrome was first described in 1966 by British neurologist J.R. Silver, who reported familiar spastic paraplegia in two unrelated British families (designated K and A) in which atrophy of the hands was the first and most marked manifestation (Silver, 1966). In these families, the presenting complaint was wasting of the small muscles of the hands and slight weakness of the shoulder muscles. The pyramidal disturbance in the lower limbs showed spastic gait and cramp. This disorder is one of the more complicated forms of hereditary spastic paraplegia (HSP) inherited in an autosomal dominant pattern.

In 2001, Patel et al. (2001a) reported that Silver syndrome is not linked to any of the identified HSP loci, and suggested that an additional locus may be responsible for this disease. Using a genome-wide screen for linkage, they subsequently demonstrated that the genetic locus of the mutation in family K mapped to chromosome 11q12–q14, and classified this syndrome as a novel HSP, spastic paraplegia 17 (SPG17) (Patel et al., 2001b). However, it has also been reported that clinical aspects of a few affected individuals in some dHMN-V pedigrees closely resemble those of Silver syndrome patients (Auer-Grimbach et al., 2000). In 2003, an Austrian group reported a detailed electrophysiological study...
of four families with Silver syndrome linked to chromosome 11q12–q14 (Windpassinger et al., 2003). The study revealed that Silver syndrome is not only caused by impairment of upper motor neurons but also by an additional peripheral motor neuropathy closely resembling clinical aspects of dHMN-V. This group also conducted haplotype analysis in an additional 16 families with phenotypes characteristic of dHMN-V or Silver syndrome. They eventually identified two different missense mutations, N88S and S90L, in the seipin gene in both diseases (Windpassinger et al., 2004), which suggested that dHMN-V and Silver syndrome are genetically identical. This gene encodes a transmembrane protein of unknown function, and loss-of-function mutations are known to be linked to a distinct recessively inherited disease, CGL type 2 (CGL2; OMIM #269700). Interestingly, both mutations, N88S and S90L, are within the N-glycosylation site of this protein, and it has been suggested that disturbance of glycosylation is associated with the pathogenesis of these diseases.

**Structure and function of the seipin gene**

The seipin gene was first identified in 2001 as a candidate gene for CGL2 by a French group (Agarwal et al., 2002). CGL, also known as Berardinelli–Seip syndrome, is a rare autosomal recessive disorder characterized by the absence of functional adipocytes (Berardinelli, 1954; Seip, 1959; Seip and Trygstad, 1996). This syndrome is classified as either CGL1 or CGL2. They are linked to two distinct genetic loci: CGL1 to the gene on chromosome 9q34 that encodes 1-acylglycerol-3-phosphate O-acyltransferase 2 (AGPAT2); and CGL2 to the seipin gene on chromosome 11q13 (Magre et al., 2001; Agarwal et al., 2002, 2004). CGL2 individuals exhibit severe lipoatrophy (a near absence of adipose tissue), insulin resistance, hypertriglyceridaemia, acromegial features and hepatomegaly (Agarwal and Garg, 2003, 2004; Fu et al., 2004). More than 20 different seipin mutations have been identified in CGL2 to date, most of which are nonsense mutations or large deletions and seem to have led to loss of function. (Magre et al., 2001; Van Maldergem et al., 2002; Agarwal et al., 2003; Ebihara et al., 2004; Jin et al., 2007). Only one missense mutation, which causes substitution of alanine at codon 212 for proline, has been reported, suggesting that this amino acid is crucial for normal structure or function of seipin. Importantly, no CGL2 mutations locate on N-glycosylation sites (NXS/T) to which a mutation of seipin-related motor neuron disease has been mapped.

A characteristic phenotype of CGL2 mutation is mental retardation, which is not seen in other CGLs such as CGL1. Notably, abnormality of motor neurons has not been documented in CGL2, whereas seipin-related motor neuron diseases are not typically described as disturbances of adipose tissue and the endocrine system. Therefore, it has been speculated that the mutation associated with CGL2 is a loss-of-function mutation in the seipin gene, and gain-of-toxic-function mutations (N88S, S90L) in this gene lead to seipinopathy.

The human seipin gene is located on the long arm of chromosome 11 and is composed of 11 exons. It has no significant homology to other known proteins. Multiple seipin transcripts of 1.8, 2.0 and 2.4 kb lengths have been identified by RNA blot analysis (Agarwal et al., 2002; Windpassinger et al., 2004). The 2.4 and 1.8 kb species are ubiquitous and could be detected in most tissues, whereas the 2.0 kb transcript is expressed selectively and at high levels in the brain and testis. A bioinformatic search of the Human GeneAtlas from the Novartis Research Foundation (http://symatlas.gnf.org/SymAtlas/) revealed that the pituitary gland also shows strong seipin expression. Recently, we assessed the distribution of seipin protein in human tissues using SCT14, a polyclonal anti-serum directed against the C-terminal peptide of human seipin (Ito et al., 2008). Immunohistochemistry revealed that seipin is selectively expressed in motor neurons in the spinal cord, in cortical neurons in the cerebral cortex, in spermatids in the testis and in the anterior lobe of the pituitary gland, which is consistent with RNA analysis. The distribution of seipin in the nervous system appears to correlate with the seipinopathies, namely the disturbance of lower and upper motor neuron function. Moreover, this finding implies that seipin may have as yet unidentified functions in spermatogenesis and the pituitary–hypothalamic system.

Originally, human seipin was predicted to be a 398-amino acid protein based on a Kozak consensus sequence, but its alignment with other species identified another Kozak sequence starting from a possible initiation site 64 bases upstream of the site identified originally. This reading frame would encode a protein of 462 amino acids. Recently, Lundin et al. (2006) showed by in vitro and by in vivo expression analysis using a full-length seipin cDNA that the predominant form of seipin is 462 amino acids long. Although the names of the two mutations associated with motor neuron diseases, N88S and S90L, are based on their positions in the 398-residue protein proposed originally, we retain these designations in the present study because they are widely known.

Alignment of the mouse and human seipin amino acid sequences showed that the region between amino acids 1 and 280 is highly conserved, suggesting that this sequence encompasses seipin's functional domain. In addition, a unique CSSS sequence at the C-terminus is also completely conserved among all species tested, suggesting that it has an important role in interacting with another molecule (Agarwal et al., 2004). Analysis using the transmembrane hidden Markov model method (http://www.cbs.dtu.dk/services/TMHMM/) predicted that seipin is a membrane protein with two transmembrane domains between residues 31–53 and residues 230–252. Previous studies using confocal analysis and density subcellular fractionation revealed that seipin is mainly localized in the ER, and a proteinase K protection assay demonstrated that seipin is an ER membrane-resident protein with a luminal loop domain and with both termini facing the cytoplasm, as shown in Fig. 1 (Ito et al., 2008).

An extensive motif search predicted that amino acids 1–280 are a possible leucine zipper domain. The secondary structure of this seipin domain is similar to those of the sterol regulatory element binding proteins (SREBPs), which are also ER-resident proteins with a leucine zipper and two transmembrane domains (Brown and Goldstein, 1997). SREBPs are dimeric basic helix-loop–helix
transcription factors that are processed and activated by site-1 protease (Ye et al., 2000). SREBPs also regulate expression of multiple genes involved in cholesterol biosynthesis and uptake. Because CGL2 is caused by a null mutation in seipin and is characterized by adipose tissue disturbances, it is likely that seipin also regulates the transcription associated with lipid metabolism.

Two recent studies based on screening of yeast deletion libraries have reported a possible function for yeast seipin (Szymanski et al., 2007; Fei et al., 2008). Their results indicate that yeast seipin is localized at the junctions between the ER and lipid droplets, so-called lipid bodies or adiposomes, and that deletion of seipin results in irregular lipid droplets (Szymanski et al., 2007). Therefore, yeast seipin seems to be a factor in assembly or maintenance of lipid droplets. In addition, fibroblasts taken from CGL2 patients can still produce lipid droplets, although they are aberrant (Szymanski et al., 2007). This study suggests that the role of human seipin may also be in the formation of lipid droplets, and failure of that process may be a molecular basis of CGL2.

During preparation of this article, Payne et al. (2008) reported important evidence that cells in which seipin was knocked down using small hairpin RNA (shRNA) failed to induce the lipogenic enzymes AGPAT2 (gene implicated in CGL1) and DGAT2, and also interfered with key adipogenic transcription factors PPARγ and C/EBPα. This evidence indicates that seipin is a gene upstream of AGPAT2 in the pathological process of lipodystrophy, and is a possible regulator of adipogenesis. However, much remains to be elucidated regarding the true role of seipin and pathogenesis of CGL. Northern blot analyses, microarray analysis and our immunohistochemistry analysis all show that expression of human seipin is low in adult adipose tissue (Magre et al., 2001; Ito et al., 2008) (http://symatlas.gnf.org/SymAtlas/), suggesting that the functional target of human seipin may reside in other tissues to regulate adipocyte differentiation via secretory factors. Moreover, CGL2 patients also exhibit other phenotypes, including insulin resistance, acromegaloïd features and mental retardation. It is less clear how human seipin contributes to the function of the endocrine and nervous systems. Considering its tissue distribution in our immunohistochemical analysis, it is possible that human seipin is a tissue-dependent and multi-functional protein.

Clinical phenotypes of seipin-related motor neuron diseases (seipinopathies)

It is known that mutations N88S and S90L in the \( N \)-glycosylation site of seipin manifest as various phenotypes, including Silver syndrome, complicated form of HSP (SPG17) and dHMN-V. In Silver syndrome, the clinical phenotype of the original K family in which mutation N88S was first identified has been reported in detail (Silver, 1966; Liu and Kaufman, 2003). The age of onset is between childhood and early adulthood, presenting in most affected individuals as both amyotrophy of the hand muscles and spasticity of the lower limbs. Sensation is usually normal, but minor sensory disturbance is exhibited in a few individuals over 60-years-old. It is notable that no cerebellar signs developed during longitudinal observation in both families. Disability of upper and lower limbs was usually not so severe that the individual could not dress, walk or carry on in the workplace. In neither family did the disorder directly contribute to death. On the other hand, dHMN-V is also characterized exclusively or predominantly by weakness and wasting of the distal muscles of the upper limbs (Auer-Grumbach et al., 2000). Main disturbances are in lower motor neurons and/or motor axons. Foot weakness and abnormalities such as a high arch (pes cavus) may also be present. People with this disorder have normal life expectancies. dHMN-V is known to be a genetically heterogeneous syndrome. Indeed, another gene, glycyl-tRNA synthetase \((GARS)\), has been identified as another candidate for the molecular basis of dHMN-V (Dubourg et al., 2006). It has been reported that mild pyramidal signs can be present in some dHMN-V families, and that their phenotypes resemble those of Silver syndrome, suggesting that clinically these diseases may represent a continuous spectrum. As mentioned previously, heterozygous mutations N88S and S90L in the seipin gene were identified in Silver syndrome and dHMN-V (Windpassinger et al., 2004). It is significant that the N88S and S90L mutations are present in both diseases, which supports the hypothesis that these diseases are indeed genetically identical. To date, more than 25 families worldwide (11 Austrian, 3 Dutch, 2 English, 2 Belgian and 1 each of Brazilian, Italian, Indian, Korean, Polish, Serbia and Swiss) having mutations in the seipin gene have been identified and described (Irobi et al., 2004; Windpassinger et al., 2004; Auer-Grumbach et al., 2005; van de Warrenburg et al., 2006; Cho et al., 2007; Dierick et al., 2008), suggesting that these may be hot-spot mutations. Recently, Dierick et al. (2008) carried out genetic analyses in a cohort of 112 patients with a clinical diagnosis of dHMN, and found that mutation in seipin is one of the most common causes of dHMN.
Detailed analysis of the clinical phenotypes caused by the N88S mutation have revealed a wide spectrum of seipin-related motor neuron disturbances in which upper and lower motor neurons and peripheral motor axons are variously affected even within the same family (Auer-Grumbach et al., 2005). Of these patients, 31.1% present the dHMN-V symptoms of predominant weakness and wasting in the small hand muscles but with normal muscular tone; 14.5% present the Silver syndrome phenotype of predominant weakness and wasting in the small hand muscles and spasticity of the lower limbs; 20% present as CMT disease, characterized by distal muscle weakness, wasting of the lower limbs and, to a lesser degree, the upper limbs, with normal muscular tone; 10% present as HSP, with spastic paraplegia in the lower limbs, but no weakness or wasting of the small hand muscles. Interestingly, penetrance is incomplete, with 20% of the individuals having the mutation showing only minor clinical signs such as mild foot deformity, minor hand atrophy and/or subclinical electrophysiological abnormalities. A small fraction (4.4%) of individuals with the mutation exhibit no clinical abnormalities and/or electrophysiological changes. Electrophysiological studies show a reduction in compound muscle action potentials (CMAPs) in the upper and lower limbs of some individuals, indicating primary axonal neuropathy. However, some cases also exhibit chronodispersion of the CMAPs and reduction of motor nerve conduction velocities (MNCVs), suggesting additional demyelination of the motor nerves. Disturbance of sensory nerve conduction is rare. Taken together, these clinical findings indicate that seipinopathies show various phenotypes but have in common the confined deterioration of upper and lower motor neurons and motor axons. How a single mutation can result in such a wide clinical spectrum is unclear. Further study is necessary to clarify whether environmental factors or other genetic variations affect the clinical phenotypes observed.

Irobi et al. (2004) reported a comparison of clinical observations of two families with the N88S or S90L mutation. Interestingly, many patients with S90L have a pronounced spastic gait and severe weakness in the lower limbs; consequently, ambulation is affected. They suggested the possibility that the S90L phenotype may be more severe than that of N88S. As shown in supplementary table, which summarizes the clinical features of the 16 families with seipinopathy (48 patients), 5 families with S90L mutation were reported in the literature. Among them, spastic gait was documented in four families (Irobi et al., 2004; Cho et al., 2007; Dierick et al., 2008). These findings are interesting considering the emerging molecular mechanism of seipinopathy. However, this finding is based on only a few pedigrees, and the biochemical characteristics of both mutations, which lie within the same N-glycosylation site, remain undistinguished on the evidence of studies reported thus far. Further study is needed to clarify genotype-phenotype correlations.

**Molecular pathogenesis of seipinopathies**

To date, there have been only a few studies (Windpassinger et al., 2004; Ito and Suzuki, 2007a; Ito et al., 2008) on the molecular mechanism of seipinopathy, and thus the pathogenesis remains unclear. Immunohistochemical analysis revealed that the distribution of seipin in the central nervous system appears to correlate with clinical manifestations of the seipinopathy (Windpassinger et al., 2004; Ito and Suzuki, 2007a; Ito et al., 2008). Seipin is expressed in motor neurons in the spinal cord and cortical neurons in the frontal lobe cortex, consistent with degeneration of lower and upper motor neurons in seipinopathy (Ito et al., 2008). A biochemical study (Ito and Suzuki, 2007a) using transfected cells demonstrated that seipin is an N-glycosylated protein that is proteolytically cleaved into N- and C-terminal fragments and polyubiquitinated. Interestingly, the N88S and S90L mutations that disrupt the N-glycosylation motif also enhance ubiquitination. Glycosylation in the ER is known to be important for the proper folding and maturation of glycoproteins, and impairment of glycosylation leads to accumulation of misfolded protein in the ER. This condition, designated ER stress, induces an intracellular signalling pathway known as the UPR (Kaufman, 1999; Liu and Kaufman, 2003; Shen et al., 2004; Hetz et al., 2006; Ron and Walter, 2007; Rutkowski and Kaufman, 2007). Severe or prolonged UPR initiates pro-apoptotic signalling cascades. Dysfunction of the protein quality control system and consequent ER stress are now thought to be associated with pathological processes in various neurodegenerative diseases (Katayama et al., 1999; Imai et al., 2000; Nakagawa et al., 2000; Ito et al., 2001, 2004; Nishitoh et al., 2002; Hetz et al., 2003; Tobisawa et al., 2003; Kikuchi et al., 2006; Lindholm et al., 2006; Turner and Atkin, 2006; Zhang and Kaufman, 2006; Pennuto et al., 2008).

Indeed, our studies have shown that both the N88S and S90L mutant seipin proteins are improperly folded, leading to accumulation of the mutant protein in the ER (Windpassinger et al., 2004; Ito and Suzuki, 2007a; Ito et al., 2008). Furthermore, transfection of mutant forms of seipin into cultured cells up-regulated well-established UPR markers BiP and CHOP, indicating that ER stress had been initiated. Moreover, both Hoechst 33342 and TUNEL staining revealed that expression of mutant protein induces apoptosis. Collectively, this is a strong evidence that seipinopathies are novel conformational diseases, and that the pathological process of these diseases is tightly associated with ER stress-mediated cell death (Fig. 2). We propose that the N88S and S90L mutations, which cause toxic accumulation in the ER resulting in ER stress, lead to motor neuron degeneration in the manner of gain-of-toxic-function of seipin; whereas, CGL2 phenotypes reflect loss of physiological function of seipin. Importantly, disturbance of motor function in CGL2 patients has not yet been reported in the literature. However, seipin is highly expressed in spinal motor neurons, suggesting the possibility of a neuronal function. Therefore, detailed clinical assessment is needed to clarify whether CGL2 is also associated with some disturbance of motor function.

Another interesting point is that cells expressing mutant seipin form inclusion bodies, although there have been no studies of autopsy or biopsy samples of seipinopathy reported to date. Although there is still no evidence of inclusion bodies in the motor neurons of patients with seipinopathy, in vitro studies have shown that ~30% of cultured cells expressing mutant
Fig. 2 Possible mechanisms of seipinopathy. Wild-type seipin is glycosylated and correctly folded by chaperones at its functional site in the ER. A small amount of wild-type seipin is misfolded, ubiquitinated and degraded by ubiquitin–proteasome system (UPS), whereas mutations that disrupt the N-glycosylation motif enhance ubiquitination and lead to the formation of inclusion bodies. These mutant seipins appear to be improperly folded, which leads to accumulation of the mutant protein in the ER and eventual neuronal cell death. On the other hand, loss-of-function mutations in the seipin gene lead to lipodystrophy.

seipin contain cytoplasmic inclusions, whereas wild-type cells do not (Windpassinger et al., 2004; Ito and Suzuki, 2007a; Ito et al., 2008). Many proteins associated with neurodegeneration, such as huntingtin, α-synuclein and Parkin, have been known to lead to formation of inclusion bodies called aggresomes, which have been well-characterized biochemically (DiFiglia et al., 1997; Klement et al., 1998; Sieradzan et al., 1999; Kopito, 2000; Waelter et al., 2001; Junn et al., 2002; Mishra et al., 2003; Taylor et al., 2003; Muqit et al., 2004; Tanaka et al., 2004). It is debatable whether the formation of inclusion bodies is pathogenic or protective in neurodegenerative diseases (Kopito, 2000; Arrasate et al., 2004; Orr, 2004). A recent study showed that inclusion bodies reduced intracellular levels of polyglutamine disease proteins such as huntingtin or ataxin 1, suggesting that they may serve a neuroprotective function (Klement et al., 1998; Arrasate et al., 2004). However, whether this is a common mechanism of protection in other neurodegenerative diseases remains unclear. Interestingly, our recent finding shows that seipin inclusions do not recruit major components of aggresomes and do not assemble at their formation sites, which are the microtubule organizing centers (MTOCs). This indicates that aggresomes have distinctive features and likely form via a different pathway. This may be a novel adaptive mechanism against toxic accumulation of misfolded protein (Windpassinger et al., 2004; Ito and Suzuki, 2007a; Ito et al., 2008). Therefore, examination of seipin inclusion bodies and the fate of cells expressing mutant seipin should provide new knowledge about the protein quality control system associated with seipinopathies and other motor neurodegenerative diseases.

Conclusions

Several studies have implicated ER stress in the form of enhanced UPR branch activity in many neurodegenerative diseases; thus, the ER stress response pathways may provide promising therapeutic targets for modifying the progression and severity of these diseases (Katayama et al., 1999; Imai et al., 2000; Nakagawa et al., 2000; Ito et al., 2001, 2004; Nishitoh et al., 2002; Hetz et al., 2003; Tobisawa et al., 2003; Kikuchi et al., 2006; Lindholm et al., 2006; Turner and Atkin, 2006; Zhang and Kaufman, 2006; Pennuto et al., 2008). However, some investigators have suggested that ER stress may not be central in the mechanism of neurodegeneration but rather participates in a minor process (Zhao and Ackerman, 2006). Indeed, the aggregated proteins associated with the majority of neurodegenerative diseases are not found within the ER, and there is no direct evidence that ER stress alone can lead to neurodegeneration in vivo models. These observations suggest that ER stress may play a small role in the neurodegeneration pathway. However, our studies indicate that the pathogenesis of seipinopathy is tightly associated with ER stress. Because tunicamycin, an inhibitor of N-glycosylation that leads to accumulation of unfolded protein in the ER, is a well-established specific inducer of ER stress, it is likely that ER stress is central to pathogenesis in seipinopathies in which mutations disrupt N-glycosylation sites. Therefore, we propose that seipinopathies should be considered as a representative of ER stress-associated neurodegenerative diseases, and studying the pathophysiology of this disorder may lead to important new insights into therapeutic approaches against ER stress.

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