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Review

The ubiquitin–proteasome system in spongiform degenerative disorders

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ABSTRACT

Spongiform degeneration is characterized by vacuolation in nervous tissue accompanied by neuronal death and gliosis. Although spongiform degeneration is a hallmark of prion diseases, this pathology is also present in the brains of patients suffering from Alzheimer's disease, diffuse Lewy body disease, human immunodeficiency virus (HIV) infection, and Canavan's spongiform leukodystrophy. The shared outcome of spongiform degeneration in these diverse diseases suggests that common cellular mechanisms must underlie the processes of spongiform change and neurodegeneration in the central nervous system. Immunohistochemical analysis of brain tissues reveals increased ubiquitin immunoreactivity in and around areas of spongiform change, suggesting the involvement of ubiquitin–proteasome system dysfunction in the pathogenesis of spongiform neurodegeneration. The link between aberrant ubiquitination and spongiform neurodegeneration has been strengthened by the discovery that a null mutation in the E3 ubiquitin–protein ligase mahogunin ring finger-1 (*Mgrn1*) causes an autosomal recessively inherited form of spongiform neurodegeneration in animals. Recent studies have begun to suggest that abnormal ubiquitination may alter intracellular signaling and cell functions via proteasome-dependent and proteasome-independent mechanisms, leading to spongiform degeneration and neuronal cell death. Further elucidation of the pathogenic pathways involved in spongiform neurodegeneration should facilitate the development of novel rational therapies for treating prion diseases, HIV infection, and other spongiform degenerative disorders.

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1. Introduction

Spongiform, or vacuolar, change describes tissue that contains numerous vacuoles that are round or oval in appearance at the light microscopic level and up to 50 μm in diameter [1–3]. These vacuoles can be either extracellular or intracellular, often displacing large

organelles such as the nucleus and mitochondria. By electron microscopy (EM), vacuoles can contain curled fragments of membranes and sometimes a fluffy or granular electron-dense material (Fig. 1B) [1,4,5]. Intramyelinic vacuoles within white matter correspond with splitting of the myelin layers [6–8]. In addition to vacuolar change, neural tissue may also exhibit microvacuolation, in which the neuropil is disrupted by numerous small vacuoles of 2 to 10 μm in diameter [2,3], or status spongiosus, where irregular cavities appear surrounded by a meshwork of glia [2,3]. Spongiform encephalopathy describes a group of diseases that exhibit vacuolar change accompanied by neural and/or glial cell death in the central nervous system. The vacuoles observed in spongiform degeneration are the result of a specific disease process and not merely the product of cell loss.

Spongiform pathology is most commonly associated with prion diseases (Fig. 1A) [1], but also occurs in the brains of patients suffering from neurodegenerative diseases (Fig. 1A) [3,4,9,10], human immunodeficiency virus (HIV) infection [11–16], and metabolic disease [17]. The extent and localization of spongiform change varies depending on the specific disease, yet each disorder shares common pathological features (Table 1). In animals, spongiform degeneration has been observed in prion disease [1], retrovirus infection (Fig. 1B) [18–20], and as a consequence of mutation in several different genes (Fig. 1B) [6,7,21–25]. The vacuolar change observed in animals shares pathological features with human spongiform encephalopathies, and

Abbreviations: AD, Alzheimer's disease; AgRP, Agouti related protein; AIDS, acquired immune deficiency syndrome; APP, amyloid precursor protein; ASPA, aspartoacylase gene; Atrn, attractin; BSE, bovine spongiform encephalopathy; CD, Canavan's spongiform leukodystrophy; CJD, Creutzfeldt–Jakob disease; *CYTB*, cytochrome b; DLBD, diffuse Lewy body disease; DUB, deubiquitinating enzyme; EM, electron microscopy; ERK, extracellular signal-related kinase; ESCRT, endosomal sorting complex required for transport; ETC, electron transport chain; FFI, fatal familial insomnia; GPI, glycosylphosphatidylinositol; GSS, Gerstmann–Sträussler–Scheinker disease; HIV, human immunodeficiency virus; Hrs, hepatocyte growth factor-regulated receptor tyrosine kinase substrate; ILV, intraluminal vesicle; *Mc(1, 3, 4)r*, melanocortin-(1, 3, 4) receptor; *Mgrn1*, mahogunin ring finger-1; MoMuLV, Moloney murine leukemia virus; α -MSH, α -melanocyte stimulating hormone; MVB, multivesicular body; NAA, N-acetyl-L-aspartate; Nrf2, NF-E2-related factor; *PRNP*, prion gene; PrP, prion protein; PrPC, cellular prion protein; PrPsc, scrapie-related prion protein; ROS, reactive oxygen species; SOD2, manganese superoxide dismutase; TSE, transmissible spongiform encephalopathy; *Tsg101*, tumor susceptibility gene 101; UPS, ubiquitin–proteasome system

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the degree and localization of spongiform change varies by infectious agent or gene mutated (Table 2). Histological comparisons of disease versus control tissue from both human and animal spongiform encephalopathies reveals increases in ubiquitinated proteins near areas of spongiform change [4,9,20,26,27], indicating that aberrant ubiquitination may be involved in the pathogenesis of spongiform degeneration.

Ubiquitination of proteins serves as a signal for a variety of cellular functions (reviewed in [28]). Ubiquitination of a substrate protein occurs via a cascade of three enzymes (an E1 ubiquitin-activating enzyme, an E2 ubiquitin-conjugating enzyme, and an E3 ubiquitin-protein ligase) and is reversible by the action of a deubiquitinating enzyme (DUB). Additional rounds of ubiquitination can result in the formation of a polyubiquitin chain through one of the seven internal lysine residues of ubiquitin. The number and location of attachment sites of mono- or polyubiquitin on a substrate protein comprises the ubiquitin signal [28,29]. A major proteolytic system in the cell is the ubiquitin-proteasome system (UPS), which degrades a majority of cytoplasmic proteins. Substrate proteins processed by the UPS are typically tagged with a polyubiquitin chain linked through Lys 48; four ubiquitins linked via K48 is the minimal signal recognized by the proteasome for substrate degradation [30]. In addition to substrate proteins, the UPS also regulates levels of the ubiquitinating enzymes. A number of E3 ligases are degraded via the UPS [31–33], thus modulating levels of ubiquitination in the cell. Since the ubiquitinating machinery is regulated by the proteasome, proteasomal impairment can result in aberrant ubiquitination of proteins in addition to accumulation of ubiquitinated proteasome substrates [34–39]. Proteasome-independent ubiquitin signals, such as monoubiquitination and K29- and K63-linked polyubiquitination, regulate endocytic trafficking, lysosomal protein degradation, DNA repair, protein aggregation and autophagic degradation, and protein localization [29,35,40–46].

Because of its versatility as a signal, changes in the ubiquitination of proteins can affect a wide range of cellular processes. This suggests that the pathogenesis of spongiform degeneration may result from dysfunction of common pathways regulated by ubiquitination in spongiform disorders with different etiologies. This review will

examine the evidence implicating several cellular pathways in spongiform degeneration and explore how these processes are connected and maintained via ubiquitination of proteins. Recent studies indicate that aberrant ubiquitination mediates the pathogenesis of spongiform change and cell death by altering normal cell function and intracellular signaling.

2. UPS dysfunction accompanies vacuolar change in spongiform encephalopathies of both humans and animals

2.1. Prion diseases

The transmissible spongiform encephalopathies (TSEs) refer to a number of fatal diseases that occur in man and animals that result in widespread vacuolation, neurodegeneration, and gliosis of the central nervous system (reviewed in [1,47]). TSEs are characterized by progressive motor difficulties, such as unsteady gait, ataxia, and tremor, accompanied by cognitive and sensory defects [1,47,48]. Human TSEs include Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker disease (GSS), kuru, and fatal familial insomnia (FFI) [1,47,48]. Animal TSEs include scrapie, bovine spongiform encephalopathy (BSE), chronic wasting disease, feline spongiform encephalopathy, and transmissible mink encephalopathy [1,47]. Spongiform areas in TSE tissues are typified by degenerating neurons that appear swollen and are associated with intracellular and extracellular membrane-bounded vacuoles containing accumulations of curled membrane fragments or other granular material [1,5]. The TSEs are known as prion diseases since prion protein (PrP) appears to be the transmissible agent in these encephalopathies, because accumulation of a disease-related conformer of PrP in the brain tissue of affected organisms occurs along with spongiform pathology [26,49,50]. Prion plaques, reminiscent of amyloid plaques observed in Alzheimer's disease (AD), are sometimes seen in the cerebellum (Fig. 1A) and other brain areas, consisting of fibrils that radiate out from a central core, giving a stellate appearance [3,26]. In advanced stages of these disorders, secondary spongiform change of the white matter may be seen [1]. Experimental models of prion diseases are

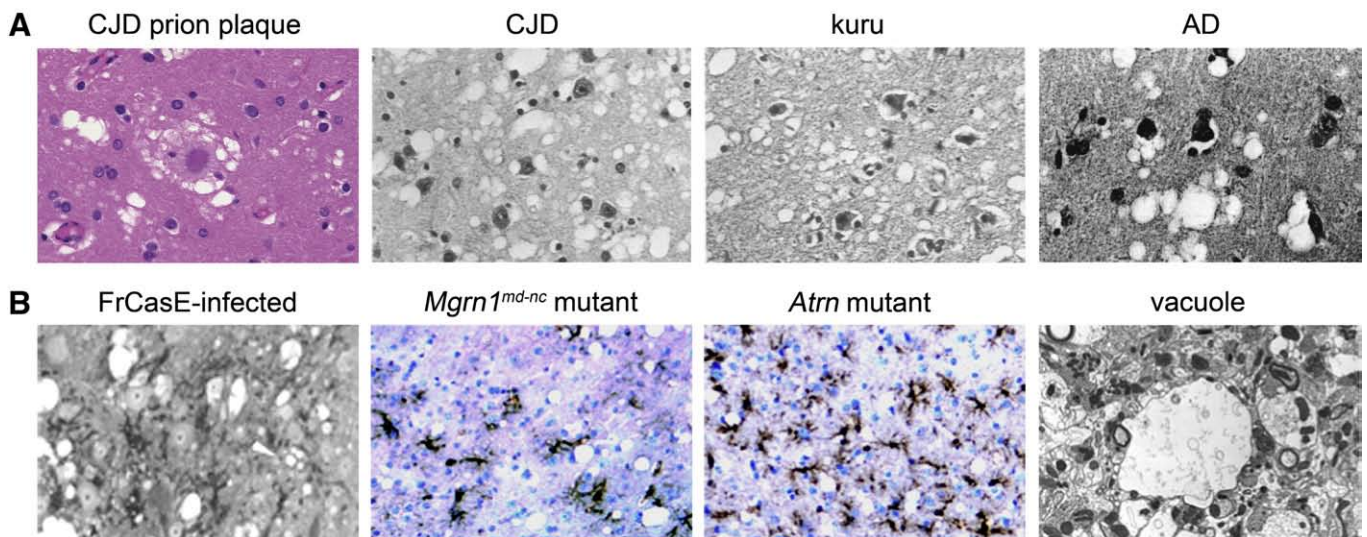


Fig. 1. Spongiform degeneration in humans and animals is characterized by vacuolar change in the central nervous system. (A) Spongiform degeneration in human diseases. Left to right, CJD prion plaque surrounded by vacuoles¹, counterstained with hematoxylin and eosin; CJD cerebral cortex², counterstained with hematoxylin and eosin; kuru cerebral cortex², counterstained with hematoxylin and eosin; and AD medial temporal lobe³, counterstained with hematoxylin and eosin. (B) Spongiform degeneration in rodents. Left to right, brainstem from mouse infected with FrCasE retrovirus⁴, immunostained with anti-FrCasE surface glycoprotein; cortex from *Mgrn1* null (*Mgrn1^{md-nc}/Mgrn1^{md-nc}*) mouse⁵, immunostained with anti-gial fibrillary acidic protein; cortex from mahogany (*Atrn^{mg3l}/Atrn^{mg3l}*) mouse⁵, immunostained with anti-gial fibrillary acidic protein; and EM of a brainstem vacuole from mahogany (*Atrn^{mg3l}/Atrn^{mg3l}*) mouse⁵. All images reprinted with permission: ¹Reprinted from Lancet Neurology [47], copyright ©2005, with permission from Elsevier. ²Reprinted from the American Journal of Pathology [1], copyright ©1972, with permission from the American Society for Investigative Pathology. ³Reprinted from Archives of Neurology [3], copyright ©1987, with permission from the American Medical Association. All Rights reserved. ⁴Reprinted from the Journal of Biological Chemistry [84], copyright ©2004, with permission from the American Society for Biochemistry and Molecular Biology. ⁵Reprinted from Science [22], copyright ©2003, with permission from AAAS.

Table 1
Spongiform degeneration in human diseases is accompanied by changes in ubiquitination

Disease	CNS location of spongiform change	Increased ubiquitination	Altered prion protein	Gliosis	Ref (s)
Creutzfeldt–Jakob disease	Grey matter of cortex, hippocampus, cerebellum, midbrain, thalamus, brain stem, motor nuclei of spinal cord	Yes	Yes	Yes	[1,2,26,49,50]
Kuru	Grey matter of cortex, cerebellum, midbrain, thalamus, brain stem, motor nuclei of spinal cord	Yes	Yes	Yes	[1,49]
Alzheimer's disease	Grey matter of temporal cortex, amygdala	Yes	Variable	Variable	[4,26,49,50]
Diffuse Lewy body disease	Grey matter of temporal cortex, amygdala	Yes	No	No	[4,9,10]
AIDS/HIV infection	Grey matter of cortex, amygdala, midbrain, thalamus; white matter tracts of spinal cord	Yes	No	Mild	[11,13–16,88]
Canavan's spongiform leukodystrophy	White matter regions of cortex, midbrain, cerebellum	ND	ND	Yes	[17]

ND, not determined.

created by injecting organisms, such as rodents or primates, with tissue from affected animals or humans. The extent of the pathology observed varies according to the strain of prion and species infected, but generally is more severe with a shorter latency than that of naturally occurring TSEs [1,5,47].

The conformational change of normal cellular PrP protein (PrP^C, cellular) into a disease-related structure (PrP^{Sc}, scrapie) is thought to initiate all TSEs. Familial forms of TSEs (GSS, FFI, and familial CJD) result from mutations in the PrP gene, *PRNP*, which presumably alter the tertiary structure of PrP [47,48,51]. In kuru, non-familial CJD, and all animal prion diseases, infection with or consumption of tissues containing PrP^{Sc} mediates the propagation of PrP^{Sc} from PrP^C. PrP^C is necessary for disease pathogenesis, since animals or cells that lack PrP^C are resistant to PrP^{Sc} infection and fail to propagate prion infectivity [52–55]. This supports the hypothesis that PrP^{Sc} acts as a template for the misfolding of PrP^C in the host's tissues (reviewed in [48,56]). This hypothesis is further supported by the observations that latency to symptom onset is dependent upon *PRNP* gene dosage [52,57] and that *PRNP* null mice carrying a hamster *PRNP* transgene are more sensitive to infection with hamster prions than with mouse prions [57,58].

Immunocytochemical staining of brain tissue from CJD or experimental murine scrapie reveals punctate ubiquitin labeling in and around areas of spongiform change as well as at the periphery of the prion plaques [26,27]. Ubiquitination of proteins in brain homogenates from scrapie-infected animals increases with disease duration as measured by enzyme-linked immunosorbent assay, while proteolytic activity of the proteasome decreases with infection [59]. These observations are supported by cDNA microarray analysis of mice infected with a strain of BSE [60], which reveals differential expression of UPS machinery genes in response to infection, including several proteasome subunits, E2s, and E3s. PrP^C has been shown to be ubiquitinated and processed by the UPS [39], while PrP^{Sc} appears to be ubiquitinated at late stages of disease when the protein is highly aggregated [59,61]. These reports indicate that dysfunction of the proteasome and the subsequent changes in protein ubiquitination resulting from proteasome impairment play a key role in prion disease pathogenesis. In addition to the UPS, other cellular pathways likely contribute to the vacuolation and degeneration of nervous tissue observed in TSEs. Increases in oxidative stress have been reported

(reviewed in [62]) and there is considerable evidence for aberration in the proteolytic processing and trafficking of PrP^C [49,63–67] and lysosomal dysfunction in TSEs [27,49,60,68,69]. These processes are regulated by both proteasome-dependent and -independent ubiquitin signaling (reviewed in [29,70,71]).

2.2. Non-transmissible neurodegenerative diseases with spongiform lesions

Progressive neurodegenerative diseases that are not characterized as TSEs, such as AD and diffuse Lewy body disease (DLBD), also exhibit spongiform change in defined brain regions [3,4,9,10]. The vacuolar change in AD and DLBD resembles TSE, although instead of widespread spongiform degeneration, vacuolation and cell loss is restricted to the medial temporal lobe and the amygdala [3,4,9]. The etiology of spongiform change is not related to PrP^{Sc}, since inoculation of laboratory animals with affected brain tissue does not result in transmission of the spongiform pathology, as it would with TSE brain tissue [4,49]. AD and DLBD brain tissue do not exhibit prion plaques or the increased PrP immunoreactivity characteristic of TSEs [4,10,26]. Interestingly, recent evidence suggests that PrP^C may modulate the processing of amyloid precursor protein (APP) [72], a protein central to the etiology of AD, since differential processing of APP contributes to the production of amyloid deposits that accumulate in AD brains [73].

Similar to TSEs, ubiquitin immunoreactivity is increased in areas near spongiform degeneration in AD and DLBD brains [9,26], and the presence of ubiquitin-positive proteinaceous inclusions is positively correlated with spongiform change [3,9]. These proteinaceous inclusions are characteristic hallmarks of neurodegenerative disease and are immunoreactive for UPS machinery in addition to ubiquitinated proteins (reviewed in [74]). Measurement of proteasome activity and ubiquitinating activity in post-mortem AD [75,76] and Parkinson's disease [77] brain tissue reveals a significant impairment of the UPS in neurodegenerative diseases. This suggests that, similar to the TSEs, cell death and spongiform change in these diseases are mediated by dysfunction of the UPS and altered cellular ubiquitin signaling. Other similarities between TSEs and neurodegenerative diseases that exhibit spongiform change include mitochondrial dysfunction and increased oxidative stress (reviewed in [78,79]), and aberrations in the endosomal trafficking of proteins (reviewed in [80]), suggesting the

Table 2
Spongiform degeneration and changes in ubiquitination in animals results from infection and gene mutation

Disease/Mutation	CNS location of spongiform change	Increased ubiquitination	Altered prion protein	Gliosis	Ref (s)
Scrapie or BSE	Grey matter of cerebellum, hypothalamus, medulla	Yes	Yes	Yes	[1]
Experimental prion infection	Grey matter of cerebellum, hypothalamus, medulla	Yes	Yes	Yes	[27]
MoMuLV-infected mouse	Grey matter of cerebellum, thalamus, brainstem, motor nuclei of spinal cord	Yes	ND	Yes	[20,82,83]
Coronavirus-infected mouse	Grey matter of cortex, hippocampus, midbrain, brain stem, spinal cord	ND	ND	Yes	[8,18]
<i>Mgrn1</i> ^{md-nc} mouse	Grey matter of cortex, cerebellum, hippocampus, midbrain, brain stem	ND	No	Yes	[22]
<i>Mahogany</i> mouse/Zitter rat	Grey matter of cortex, cerebellum, midbrain, hypothalamus, medulla	Yes	No	Yes	[21,22,25,106,107,113,117]

ND, not determined.

involvement of both proteasome-dependent and proteasome-independent ubiquitination in the pathogenesis of these diseases.

2.3. Viral-mediated diseases

Spongiform pathology has been reported in central nervous system tissue from patients with acquired immune deficiency syndrome (AIDS) resulting from HIV infection. Approximately one-fifth of patients have white matter vacuolation of the spinal cord [12–14], and a smaller percentage of patients have spongiform change in cortical areas [11,13,16]. Animal models of HIV infection have more extensive spongiform pathology. Macaques infected with simian immunodeficiency virus [19] and mice infected with a number of different retroviruses [8,18,20,81,82] display spongiform degeneration of either grey or white matter and exhibit motor deficits. Differences in the localization and extent of spongiform pathology depend upon the strain of virus used and the cell type(s) that each virus strain infects [82,83]. The retroviral env protein appears to confer many of the differences between retroviral strains [84–87]. Experimental manipulation of env to create chimeric env proteins from different strains of a mouse retrovirus has revealed that env plays a large role in conferring neurovirulence [84]. Differential post-translational processing of env by different cell types in mouse brain affects viral production and presence of spongiform pathology in a given brain region [86]. Furthermore, transgenic expression of a retroviral env protein by itself is sufficient to produce spongiform pathology in mice [85], so production of viral particles per se does not lead to vacuolation and cell death.

Similar to human neurodegenerative diseases, brain areas exhibiting spongiform change display no increase in PrP immunoreactivity [14], but do show an increase in ubiquitin staining in areas adjacent to those displaying vacuolar change [20,88]. Infection of mice with the *ts1* strain of Moloney murine leukemia (MoMuLV) retrovirus results in spongiform degeneration with Lewy body-like cellular inclusions near areas of spongiform change [20], suggesting that viral infection may promote protein aggregation. One pathway by which retroviruses could increase protein aggregation is by disrupting UPS function [34,38,89], similar to the UPS dysfunction and ubiquitin-positive proteinaceous inclusions associated with neurodegenerative diseases or the decrease in proteasome activity and presence of ubiquitinated, aggregated PrP^{Sc} observed in TSEs.

The importance of the UPS in the onset of spongiform pathology in retrovirus infection models is highlighted by a recent study [90], which examined the effect of different ubiquitin mutant transgenes in mice infected with *ts1* MoMuLV. Transgenic mice that express K48R ubiquitin, which is capable of forming polyubiquitin chains of any linkage except K48, survive longer and demonstrate delayed onset of spongiform pathology compared to wild-type controls after *ts1* infection. K63R ubiquitin transgenic mice, whose cells can produce polyubiquitin chains of any linkage except K63, had similar disease onset and survival as wild-type ubiquitin mice [90]. These results suggest that K48-linked polyubiquitination and proteasomal degradation may be involved in the neurodegenerative process and onset of spongiform pathology caused by retroviral infection in mice and humans. However, since elimination of K48-mediated ubiquitination only delayed and did not prevent spongiform pathology and cell loss, proteasome-independent ubiquitin signaling may also be utilized by the cellular processes that contribute to spongiform degeneration. These processes likely include oxidative stress pathways [91,92], and functions of the endosome–lysosome system [93–97].

2.4. Spongiform degeneration caused by genetic mutations in humans and rodents

2.4.1. Aspartoacylase (ASPA)

A human metabolic disease, Canavan's spongiform leukodystrophy (CD), results in epilepsy and spongiform degeneration of white matter

[17]. This disease results from mutation in the *aspartoacylase* (ASPA, also known as N-acetyl-L-aspartate aminohydrolase) gene, which encodes the enzyme responsible for catabolizing N-acetyl-L-aspartate (NAA). Patients with CD have a buildup of NAA levels in the brain, as well as increases of NAA in the blood and urine [17]. A rodent form of CD, the *Tremor* rat, harbors a spontaneous deletion in *ASPA*, resulting in no protein product and increased NAA levels in the brain [23]. Animals exhibit seizure-like activity and spongiform degeneration of both white and grey matter. Viral gene transfer of *ASPA* into the *Tremor* rat ameliorates the seizures but does not attenuate spongiform pathology [98], suggesting that vacuolation and degeneration is not a direct result of increased NAA levels, but possibly signaling pathways initiated by an increase in NAA. Injection of NAA into rats can produce seizures [23], and NAA treatment of rat cortical tissue increases markers for oxidative stress [99], which indicates that neuronal excitability and oxidative stress signaling contribute to spongiform degeneration resulting from increased brain NAA levels in CD. Oxidative stress can modulate a number of cellular pathways, including gene transcription [100–102], receptor tyrosine kinase signaling [103], and apoptosis [79,104]. Increased oxidative stress is known to alter levels of ubiquitination in the cell, as well as change transcription of several UPS genes [102,105]. Evidence for increased oxidative stress in CD and the *Tremor* rat indicates that, in addition to oxidant signaling pathways, the pathogenesis of spongiform change may be mediated by changes in ubiquitination.

2.4.2. *Attractin* (*Atrn*)

The *mahogany* mouse [21,106] and the *Zitter* rat [25,107] both exhibit spongiform pathology in the brain, body tremor, and changes in hair color and texture. Both rodents harbor spontaneous mutations in the *attractin* (*Atrn*) gene [21,106,108,109], which encodes a protein known to regulate coat color in rodents by modulating the melanocortin signaling pathway [108,110,111]. Signaling via melanocortin receptors (Mc1r, Mc3r, and Mc4r) is activated by the ligand α -melanocyte stimulating hormone (α -MSH) and antagonized by the inverse agonists Agouti and Agouti-related protein (AgRP) [108,110]. The melanocortin-1 receptor (Mc1r) is a key regulator of pigment production in hair follicle melanocytes (Fig. 2A), while in brain cells, body mass and energy homeostasis are regulated via Mc3r and Mc4r (Fig. 2B) [110].

Atrn is a type I transmembrane protein that acts as an accessory receptor for Agouti, but not AgRP [108,110,111]. *Atrn* may exert its effects in the melanocyte by either extending Agouti inhibition of Mc1r or via an unknown intracellular signaling cascade. Mutation of *Atrn* increases Mc1r signaling, resulting in animals with a darker coat [108,111]. In the neuron, *Atrn* may or may not act via an unknown signaling pathway to modulate Mc3r and Mc4r signaling; mutation of *Atrn* results in leaner mice [106,110], but has no significant effect on food intake [106], indicating that *Atrn* may impact energy homeostasis signaling, possibly via a downstream effector of Mc3r/Mc4r or a separate pathway. *Atrn* may also be involved in cell survival signaling in the neuron (Fig. 2B), since small interfering RNA-mediated knockdown of *Atrn* exacerbates cell death in response to proteasome or mitochondrial inhibition [112] and one study [109] suggests that *Atrn* plays a role in antioxidant signaling via the extracellular signal-related kinase (ERK)-mediated cell survival pathway. *Zitter* rats exhibit age-related neurodegeneration of substantia nigra dopaminergic neurons [113], which are sensitive to changes in oxidative stress. These neurons display increased ubiquitin immunoreactivity and, by EM, abnormalities of mitochondria and endocytic structures [113]. *Mahogany* mice are also reported to have mitochondrial abnormalities, including functional deficits and increased oxidative stress [114]. These findings indicate that changes in oxidant signaling, ubiquitination, and endocytic trafficking may all contribute to the spongiform pathology seen in these mutant mice.

2.4.3. Mahogunin ring finger-1 (*Mgrn1*)

The link between perturbations in ubiquitination and human spongiform disorders is especially salient given that loss of the E3 ubiquitin–protein ligase mahogunin ring finger-1 (*Mgrn1*) [22,115] results in progressive spongiform degeneration in mice. Null mutation of *Mgrn1* (*Mgrn1^{md-nc}*) produces a very similar phenotype to *Atrn* mutations, including widespread spongiform degeneration, body tremor, and dark coat [22,111,116]. Despite the similarity between *Mgrn1^{md-nc}* and *mahogany* phenotypes, biochemical interaction between *Mgrn1* and *Atrn* has not been established. *Atrn* protein levels are normal in *Mgrn1^{md-nc}* mice [22], suggesting that either *Atrn* is not a substrate for *Mgrn1*-mediated ubiquitination or *Mgrn1*-mediated ubiquitination of *Atrn* is a proteasome-independent signal. Ectopic expression of *Atrn* does not rescue the *Mgrn1* null mutation, indicating that *Mgrn1* function is genetically downstream of *Atrn* function [22]. Like *Atrn*, *Mgrn1* is also involved in the melanocortin signaling pathway, although precisely how *Mgrn1* modulates melanocortin receptor signaling is unknown [108,110,111]. One possibility is that *Mgrn1* ubiquitinates the receptors or the downstream effectors of *Mc1r/Mc3r/Mc4r* (Fig. 2). Another possibility is that *Mgrn1* acts by ubiquitinating components of an unknown *Atrn* signaling pathway. Identification of *Mgrn1* substrates will help elucidate how *Mgrn1* regulates melanocortin signal transduction.

The neurodegeneration resulting from *Mgrn1* null mutation indicates that *Mgrn1*, like *Atrn*, may also participate in cell survival signaling. The widespread vacuolation of brain tissue seen in these mutant mice is very similar to the pattern observed in TSEs (Table 2). However, unlike TSEs, the brain tissue of these mice does not exhibit increased PrP immunoreactivity or protease-resistant PrP^{Sc} [22,117]. While PrP is ubiquitinated [39], *Mgrn1* does not ubiquitinate PrP [22], implying that the spongiform degeneration seen in *Mgrn1^{md-nc}* is not directly related to prion protein processing and toxicity. We recently identified the endosomal sorting protein, tumor susceptibility gene 101 (*Tsg101*), as a substrate of *Mgrn1* E3 ligase [118], associating dysfunction of endosomal ubiquitin signaling to the pathogenesis of spongiform change in *Mgrn1* null mice. A recent report [114] describes mitochondrial dysfunction and increased levels of oxidative stress in the *Mgrn1^{md-nc}* mutant mice, suggesting that oxidative stress

may also play a role in the spongiform degeneration observed in these mice.

3. Proteasome-dependent and -independent ubiquitin signaling regulates the cellular response to stress and activation of cell death pathways in spongiform degeneration

3.1. Oxidative stress can inhibit the UPS and contribute to apoptotic cell death in spongiform degeneration

The aberrant ubiquitination observed in spongiform encephalopathies is indicative of increased cellular stress and dysfunction of the UPS. Altered ubiquitin signaling can affect a number of cellular pathways, including those that control cell survival. Programmed cell death via apoptosis is regulated extensively by the UPS (reviewed in [119]), since a number of pro- and anti-apoptotic proteins are E3 ligases and their substrates [120–126]. Mitochondria are also mediators of apoptotic cell death; leakage of mitochondrial proteins into the cytoplasm is a potent proapoptotic factor (reviewed in [127]). In addition, mitochondria can be acted upon by signals both inside and outside the cell to promote or circumvent apoptosis [122,128–130].

Activities of the mitochondria and proteasome are linked, since inhibition of the proteasome can impair mitochondrial function [131–134] and mitochondrial impairment leads to decreases in proteasome activity and upregulation of ubiquitinating enzymes [131,135–138]. Recent evidence indicates that the UPS may be critical for the maintenance of mitochondrial fusion and fission machinery [139–143], which is important for sustaining mitochondrial function and health. These studies indicate that cross-talk between the mitochondria and UPS regulates apoptotic signaling, so dysfunction of either the proteasome or mitochondria can initiate mitochondria-mediated apoptosis and result in cell death [144].

One major source of cellular stress is the oxidation of proteins, lipids, and nucleic acids by reactive oxygen species (ROS) [145,146]. ROS are generated by the function of the mitochondrial electron transport chain (ETC), but are normally controlled by cellular antioxidant systems [147–150]. Increases in cellular ROS, resulting from impairment of the ETC or loss of protein antioxidants, can easily

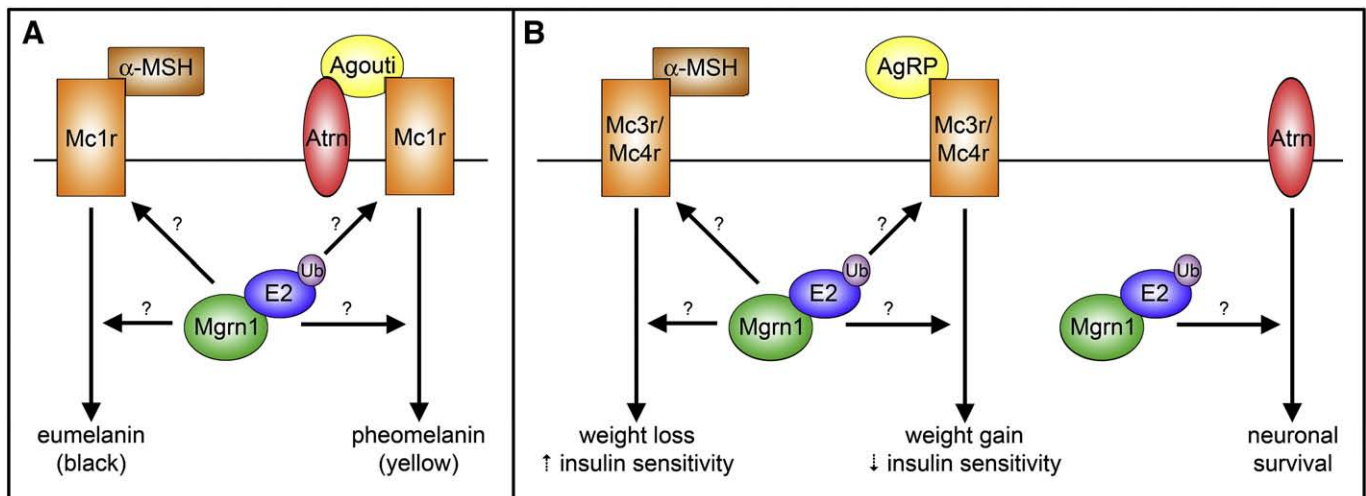


Fig. 2. Pathways by which *Mgrn1*-mediated ubiquitination could modulate melanocortin receptor regulation of pigment production and energy metabolism. (A) *Mc1r* signaling in hair follicle melanocytes regulates coat color. Activation of *Mc1r* by the ligand α -MSH results in production of the black pigment eumelanin, while suppression of *Mc1r* by the inverse agonist *Agouti* promotes the production of the yellow pigment pheomelanin. *Mgrn1* may ubiquitinate either *Mc1r* or a downstream target, complementing *Agouti* signaling in non-mutant mice. Reduction or loss of *Mgrn1*-mediated ubiquitination in *Mgrn1^{md-nc}* mice would then stimulate production of eumelanin, producing a darker coat. (B) *Mc3r* and *Mc4r* signaling in hypothalamic neurons modulates insulin sensitivity, regulating energy homeostasis and body mass. Receptor activation by α -MSH increases insulin sensitivity, promoting leaner body mass, and receptor inhibition by *AgRP* decreases insulin sensitivity, promoting obesity. In wild-type mice, *Mgrn1*-mediated ubiquitination of possibly either the receptors or a downstream target could modulate melanocortin signaling in hypothalamic neurons, while in *Mgrn1* null mice, a reduction or loss of *Mgrn1* activity could then alter energy homeostasis. The role, if any, of *Atrn* in hypothalamic melanocortin signaling is unclear, although *Atrn* may regulate neuronal survival via an unknown pathway, which could be modulated by *Mgrn1*-mediated ubiquitination.

overwhelm cellular defenses [151–153]. ROS damage to mitochondria can lead to additional ROS release and initiation of apoptosis [154–156]. ROS damage to proteins can change protein conformation, alter enzyme activity, and promote aggregation, necessitating clearance of damaged proteins by the cell's proteolytic machinery, usually the UPS (reviewed in [145,157]). Proteasome activity increases in cells exposed to mild oxidative stress [138], presumably to handle increased cellular levels of proteasome substrates. However, high levels of ROS can oxidatively modify the proteasome, decreasing its activity [135] and impairing the cell's ability to remove damaged proteins, resulting in increases of ubiquitinated and aggregated proteins [34,38,105,158,159]. In addition to oxidative modification of cellular components, low levels of ROS can serve as an intracellular signal for kinase activation and gene transcription (reviewed in [103,160]).

Two models of oxidative stress, manganese superoxide dismutase (SOD2) and NF-E2-related factor (Nrf2) knockout mice, display spongiform degeneration [99,156,6,7], highlighting a role for oxidant signaling and cellular stress in the pathogenesis of spongiform encephalopathies. SOD2 is a cellular antioxidant and Nrf2 is an oxidant-responsive transcription factor; knockdown of either of these proteins impairs cellular defenses against ROS. Furthermore, impairment of mitochondrial function by mutation of ETC subunits can result in increased ROS and spongiform degeneration: missense mutation of the ETC complex III subunit, cytochrome b (*CYTB*), produces spongiform degeneration that resembles CD in dogs, which is accompanied by impairment of ETC activity and increased ROS [24], while mutations of the ETC complex IV subunit *Vla* cause decreases in mitochondrial function, resulting in neurodegeneration and vacuolation of brain tissue in fruit flies [161]. These examples indicate that mitochondrial dysfunction and increased ROS can activate pathways that result in both vacuole formation and cell death.

Increases in markers for oxidative stress and apoptotic cell death have been reported for prion diseases [62,162], neurodegenerative diseases [78,79,151], retroviral infection [91,92], a mouse model of CD [99], *Zitter* rat [109,163], and both *mahogany* and *Mgrn1^{md-nc}* mutant mice [114]. The role of oxidative stress in promoting spongiform pathology is emphasized by studies that demonstrate a protective effect of antioxidants [91,99,163,164]. Treatment of *ts-1* MoMuLV-infected mice with antioxidants delays onset and decreases severity of spongiform pathology [91]. In addition, antioxidant treatment decreases apoptotic cell death of cells from *Zitter* rats [163], *SOD2^{-/-}* mice [164], and cells exposed to NAA [99]. Because antioxidants do not prevent spongiform pathology, oxidative stress signaling is probably only one of multiple pathways involved in the pathogenesis of spongiform disorders.

Increased oxidative stress may contribute to spongiform pathology via several pathways (Fig. 3). Central to these pathways is the interdependence of normal function of the proteasome and mitochondria. Regardless of the precipitating factor(s) specific to each of the spongiform degenerative disorders, once proteasome function is compromised, mitochondria can become impaired and vice versa [131–138]. An escalation of cellular stress could occur, since not only does a decrease in proteasome activity result in protein aggregation [34,89,158], but protein aggregates have been experimentally shown to directly inhibit the proteasome [165,166], likely decreasing proteasome activity further. Increases in ROS can accelerate this process by promoting both aggregation and proteasomal impairment [135,137,157,167], contributing to mitochondrial dysfunction and additional ROS production. This interplay between ROS levels, mitochondrial dysfunction, proteasome impairment, and protein aggregation could result in the release of proapoptotic factors from mitochondria and initiation of apoptosis in neurons, possibly contributing to the cell death characteristic of spongiform degeneration. Proteasomal dysfunction can also result in aberrant ubiquitination of proteins, since many ubiquitinating enzymes are regulated by

proteasomal degradation [31–34,89,158], which can alter signaling in many cellular pathways due to changes in the amount, activity, conformation, and/or localization of proteins regulated by ubiquitination [29,35,40–46]. Impairment of the proteasome could also promote a shift to lysosomal/autophagic degradation of proteins in the cell (reviewed in [168,169]), which is discussed in the following section.

In TSEs, increased ROS may promote the conversion of PrP^C into PrP^{Sc}. Experimental treatment of recombinant PrP^C with ROS in vitro generates oxidized PrP that tends to aggregate, is protease-resistant, and is capable of templating itself, much like PrP^{Sc} [167]. Aggregation of PrP^{Sc} can inhibit the proteasome [166] and PrP aggregation can activate apoptotic pathways [170]. Oxidative stress signaling via the ERK pathway is influenced by both PrP^C [53] and Atrn [109]. Loss of function of either protein via modification or mutation results in a decrease in activated ERK and an increase in apoptotic cell death in response to oxidative stress [53,109]. PrP null mice have increased levels of oxidized and ubiquitinated proteins in their brains and decreased proteasome activity, supporting a role for PrP^C in the cellular regulation of oxidative stress [171] and suggesting that increases in protein aggregation and decreased proteasome activity may result from loss of PrP^C function in TSEs.

Mitochondrial-mediated apoptosis likely contributes to neurodegeneration in retroviral infection, since overexpression of the antiapoptotic protein Bcl-2 in mice increases survival and decreases severity of vacuolar change after *ts-1* MoMuLV infection [172]. Atrn and *Mgrn1* may play a more direct role in maintenance of mitochondrial function, since both *Mgrn1^{md-nc}* and *mahogany* mice exhibit mitochondrial defects in addition to spongiform change [114]. Whether caused by aggregated PrP, virus, or gene mutation, impairment of the UPS and altered ubiquitination in spongiform disorders could result in mitochondrial dysfunction, release of ROS, increase of cellular stress, and activation of apoptosis (Fig. 3). However, both antioxidants and antiapoptotic factors fail to prevent spongiform degeneration, indicating that other cellular pathways contribute to cell death and vacuole formation in spongiform pathogenesis.

3.2. UPS dysfunction and increased cellular stress can lead to autophagic cell death in spongiform disorders

Accumulation of damaged or misfolded proteins that cannot be processed by the UPS can inhibit proteasome function [165] and increase cellular stress. Increased levels of cellular stress may alter ubiquitin signaling, shuttling proteins towards proteasome-independent degradation when the UPS is overwhelmed or compromised [168,173]. We and others have shown that aggregation of proteins into a specialized structure called the aggresome is mediated by K63-linked polyubiquitination [37,174]. Sequestration of harmful molecules into an aggresome minimizes their toxicity and allows the cell to degrade them in bulk via autophagy (reviewed in [175]). Autophagy (“self-eating”) is a cellular process whereby cytoplasmic contents, organelles, or cells are surrounded by membranes (forming an autophagosome) and delivered to the lysosome for degradation (reviewed in [176]). Autophagy is activated in response to impairment of the UPS [173], increased cellular stress [168], and ROS signaling [177–179]. Autophagy must be tightly regulated by the cell; autophagy dysfunction [180,181] or prolonged activation of autophagy [182] can result in autophagic cell death.

The membrane-bound vacuoles observed in spongiform encephalopathies resemble autophagic vacuoles, leading to the hypothesis that spongiform degeneration results from autophagic cell death [5,183]. Accumulation of autophagosomes has been reported for CJD [184], experimental prion disease [183,185], neurodegenerative disorders [186,187], retrovirus infection [20], and in *Zitter* rat [113]. This increase in autophagic vacuoles may result from the cell trying to rid itself of unwanted proteins that are accumulating due to impairment

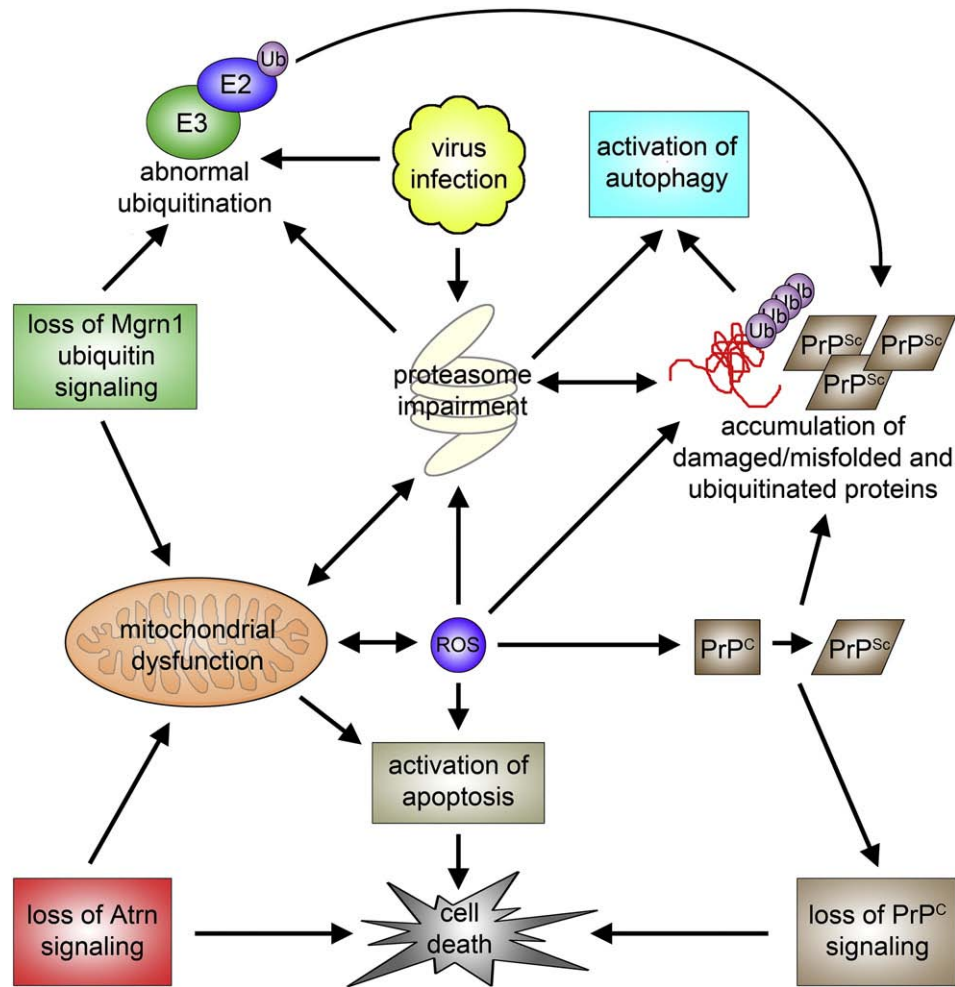


Fig. 3. Potential mechanisms by which impaired proteasome function and increased oxidative stress in spongiform encephalopathies could result in cell death. Impairment of the proteasome by protein aggregates, perturbations in ubiquitin signaling, mitochondrial dysfunction and/or increased cellular ROS can result in increases in proteasome substrates and protein aggregates, alterations in ubiquitination, and apoptotic and/or autophagic cell death via mitochondrial dysfunction and prolonged or aberrant activation of autophagy. Impairment of mitochondrial function by loss of Mgrn1 or attractin signaling, proteasomal dysfunction, or increased cellular ROS can activate apoptosis, increase cellular ROS levels, and inhibit the activity of the proteasome. Increased oxidative stress can promote protein aggregation, which can inhibit the proteasome and activate autophagy, impair the functions of both the mitochondria and the proteasome, and activate apoptosis.

of the proteasome and protein damage due to increased ROS (Fig. 3) [104,168,182].

Alterations in activation of autophagy can affect mitochondrial health, as damaged mitochondria are degraded via autophagy [188]. Ubiquitination of outer mitochondrial membrane components tags mitochondria with ubiquitin and serves as an autophagic degradation signal in several cell types [189–191], suggesting that altered ubiquitination may impair the removal of mitochondria by autophagy. Increases in cellular ROS levels by dysfunctional mitochondria can damage lysosomes, while impairment of lysosomal function can cause accumulation of damaged mitochondria from reduced mitochondrial autophagy (reviewed in [192]). Impairment of proteasomal and lysosomal degradative pathways can lead to the activation of both apoptotic and autophagic cell death. In addition, prolonged impairment of the proteasome can result in increased amounts of lipofuscin, a nondegradable material in lysosomes, which is a marker for lysosomal dysfunction [193]. As illustrated in Fig. 3, the cell must maintain a delicate balance between normal functions of the UPS and mitochondria to avoid a buildup of protein aggregates and ROS that can activate either autophagy or apoptosis. The mitochondrial deficits, increased oxidative stress, and neurodegeneration observed in *mahogany* and *Mgrn1^{md-nc}* mice [114] likely indicate an upset of this balance.

Although apoptosis and autophagic cell death are different pathways that result in cell death, experimental evidence supports both

processes contributing to cell death in response to mitochondrial inhibition and increased ROS [104]. Therefore, neurodegeneration in spongiform encephalopathies could be the result of activation of both apoptotic and autophagic cell death pathways. The intracellular and extracellular vacuoles that characterize spongiform change in grey matter may originate from incompletely degraded and/or incorrectly trafficked autophagosomes. Exocytosis of autophagosomes could contribute to extracellular vacuoles observed in spongiform tissue; autophagic cell death could also produce extracellular vacuoles, since undegraded autophagosomes could persist in the neuropil after loss of the cell that originally contained them. The neurodegeneration and vacuolation of tissue in spongiform disorders may result from activation of both apoptotic and autophagic cell death pathways by proteasome impairment and/or changes in ubiquitination that alter cellular signaling.

4. Ubiquitin-dependent regulation of intracellular trafficking is perturbed in spongiform encephalopathies

Trafficking of proteins via the endosomal pathway into the lysosome comprises a major proteolytic system for membrane proteins in the cell. In addition to regulating protein levels, endocytic trafficking also modulates cell surface receptor signaling [70,71,194]. Modification of endocytic cargo proteins and the endocytic sorting

machinery by ubiquitination (typically mono- or multi-monoubiquitination) regulates intracellular trafficking [29,43,46,195,196]. Because the UPS controls the levels of the E3 ligases and DUBs that modulate endocytic trafficking [35,118,197], the proteasome and lysosome degradative systems are not independent from one another. Proper regulation of endocytic sorting is also necessary for autophagic degradation [198] and cellular health; mutations in endosomal sorting proteins have been linked to autophagy dysfunction and neurodegeneration [199].

Sorting at the early endosome is accomplished by the endosomal sorting complexes required for transport (ESCRTs, numbered 0, I, II, and III). ESCRTs are responsible for directing lysosome-bound cargo into endosomal intraluminal vesicles (ILVs), forming a structure called the multivesicular body (MVB) (reviewed in [46]). The MVB/late endosome can then fuse with the lysosome, delivering the ILVs for degradation. The MVB can also fuse with the plasma membrane, releasing the ILVs as exosomes (reviewed in [200]), which may function in intercellular signaling. Intracellular trafficking and lysosomal degradation is perturbed in TSEs [26,27,63,68,69,201,202], AD [80], retroviral infection [93,94,97], cells lacking *Mgrn1* [118], and *Zitter* rat [113].

PrP^C is a glycosylphosphatidylinositol (GPI)-anchored glycoprotein that primarily is trafficked via the secretory pathway to the plasma membrane, although populations appear in the endocytic pathway [65,67] and cytoplasm [39,66]. PrP membrane association is necessary for prion disease, since mice expressing PrP^C missing the GPI anchor are resistant to clinical disease after infection with scrapie, similar to PrP knockout mice, despite having high levels of PrP^{Sc} and amyloid plaques in their brains [202]. This indicates PrP^{Sc} toxicity is likely dependent upon its localization to the plasma membrane and/or endocytic system. The conformational change from PrP^C to PrP^{Sc} probably depends upon a number of factors, but abnormal trafficking to an acidic compartment like the lysosome or MVB may contribute to the propagation of protease-resistant PrP [64]. In fact, subcellular fractionation of scrapie-infected mouse brain [63] reveals that protease-resistant PrP (PrP^{Sc}) accumulates in fractions that also contain markers for the late endosome and lysosome. This is in contrast to protease-sensitive PrP (PrP^C), which is prevalent in lighter fractions representing early endosomes [63], indicating that the conversion of PrP^C into PrP^{Sc} may alter the trafficking and localization of PrP in the cell. PrP is known to be released extracellularly via exosomes [203], so changes in PrP trafficking could modify the amount of prion protein released by exosomes. Altered trafficking of PrP^{Sc} compared to PrP^C would also modify intracellular signaling (Fig. 4), which could contribute to spongiform degeneration [71].

Retroviruses utilize the endosomal sorting machinery for viral budding (reviewed in [204,205]), perturbing normal endocytic sorting and trafficking [94,206]. This hijacking of the endocytic machinery occurs because viral gag proteins can disrupt the interaction between the ESCRT-0 protein hepatocyte growth factor-regulated receptor tyrosine kinase substrate (Hrs) and the ESCRT-I protein, Tsg101 [87,94]. Binding of gag to Tsg101 recruits the remaining ESCRT proteins to either the MVB or the plasma membrane for assistance in viral budding [204,206], disrupting sorting of normal endocytic cargo (Fig. 4). Trafficking defects of endocytic cargo in HIV-infected cells can be rescued by overexpression of Tsg101 [94], suggesting that these defects are dependent upon competition between Hrs and gag for a limited amount of ESCRT-I. The interaction of retroviral gag proteins with Tsg101 is dependent upon membrane association and monoubiquitination of gag [93,207]. Alterations in trafficking by viral infection affect intracellular signaling, and increased release of exosomes alters intercellular signaling. Increases in exosome release are observed with retroviral infection, since viral particles produced at the MVB are then released via exosomes [96,208]. Extracellular PrP release is increased in cells that are infected with both PrP^{Sc} and MoMuLV [209]. Thus, retroviral infection

alters normal intracellular trafficking and can impair lysosomal degradation of proteins (Fig. 4).

Our recent identification of Tsg101 as a substrate for the E3 ubiquitin ligase *Mgrn1* has emphasized the role of endocytic trafficking in spongiform disorders [118]. Like other sorting proteins, Tsg101 activity is regulated by ubiquitination [196]. We identified an interaction between *Mgrn1* and Tsg101 and found that *Mgrn1* multi-monoubiquitinates Tsg101. Small interfering RNA-mediated knock-down of *Mgrn1* results in enlargement of the endocytic compartment and impairment of the lysosomal degradation of epidermal growth factor receptor [118]. This indicates that loss of *Mgrn1*-mediated ubiquitination of Tsg101 in *Mgrn1*^{md-nc} mice impairs sorting at the endosome, preventing endocytic cargo from being degraded by the lysosome, as well as potentiating receptor signaling (Fig. 4). Impairment of lysosomal degradation, like proteasomal inhibition, results in accumulation of proteins destined for degradation [210]; this accumulation increases cellular stress, activating cell death pathways [104,173]. Impairment of sorting at the endosome also results in enlargement of endocytic structures [46,69,80,118]; enlarged endosomes could also contribute to the membrane-bounded vacuoles observed in spongiform diseases in grey matter. In white matter, perturbation of the endocytic trafficking of myelin proteins and lipids (reviewed in [211]) could contribute to intramyelinic vacuoles by interfering with processes necessary for the maintenance of myelin sheaths. Evidence of endocytic trafficking dysfunction in TSEs, retroviral infection, and *Mgrn1* null mice reveals that accumulation of lysosomal substrates either through impairment of ESCRT sorting or a shift towards exosome release could play a role in the pathogenesis of spongiform disorders (Fig. 4). Diversion of lysosomal substrates from the lysosome can alter intracellular signaling, which can then activate both apoptotic and autophagic cell death pathways, likely causing accrual of autophagosomes and increased cell death, which could result in spongiform change and neurodegeneration.

5. Conclusions

Spongiform degeneration is the hallmark of prion diseases, yet this pathology is not specific to prion protein. Spongiform disorders of different etiologies share dysfunction of common processes, indicating that multiple factors act via the same cellular pathways to produce spongiform pathology. Perturbation of ubiquitination is seen in brain areas exhibiting spongiform change, suggesting a role for proteasome dysfunction and altered ubiquitin signaling in these disorders. The discovery that loss of the E3 ligase *Mgrn1* in the *Mgrn1*^{md-nc} mouse results in spongiform neurodegeneration supports the idea that ubiquitination modulates the pathways involved in the onset of spongiform change. Alterations in ubiquitin signaling modulate cellular processes by both proteasome-dependent and proteasome-independent mechanisms. Changes in ubiquitination resulting from PrP^{Sc}, viral infection, environmental factors, aging, or gene mutation in these encephalopathies could impair protein degradation via both the proteasome and the lysosome. Accumulation of proteasomal and lysosomal substrates could then increase levels of cellular stress, activating cell death signaling.

Identification of the pathways involved in spongiform neurodegeneration has been aided by a number of genetic mutants that exhibit spongiform change. Mutations in *ASPA*, *SOD2*, *Nrf2*, and *CYTB* emphasize a role for mitochondrial dysfunction and increased ROS in the onset of spongiform degeneration. Mitochondrial dysfunction and oxidant signaling are important activators of apoptotic cell death, which could produce the neurodegeneration seen in spongiform encephalopathies. Both oxidant and ubiquitin signaling can activate autophagy, which could be a contributor to spongiform vacuole formation. Spongiform vacuoles share many characteristics with autophagosomes and accumulation of autophagic vacuoles has been

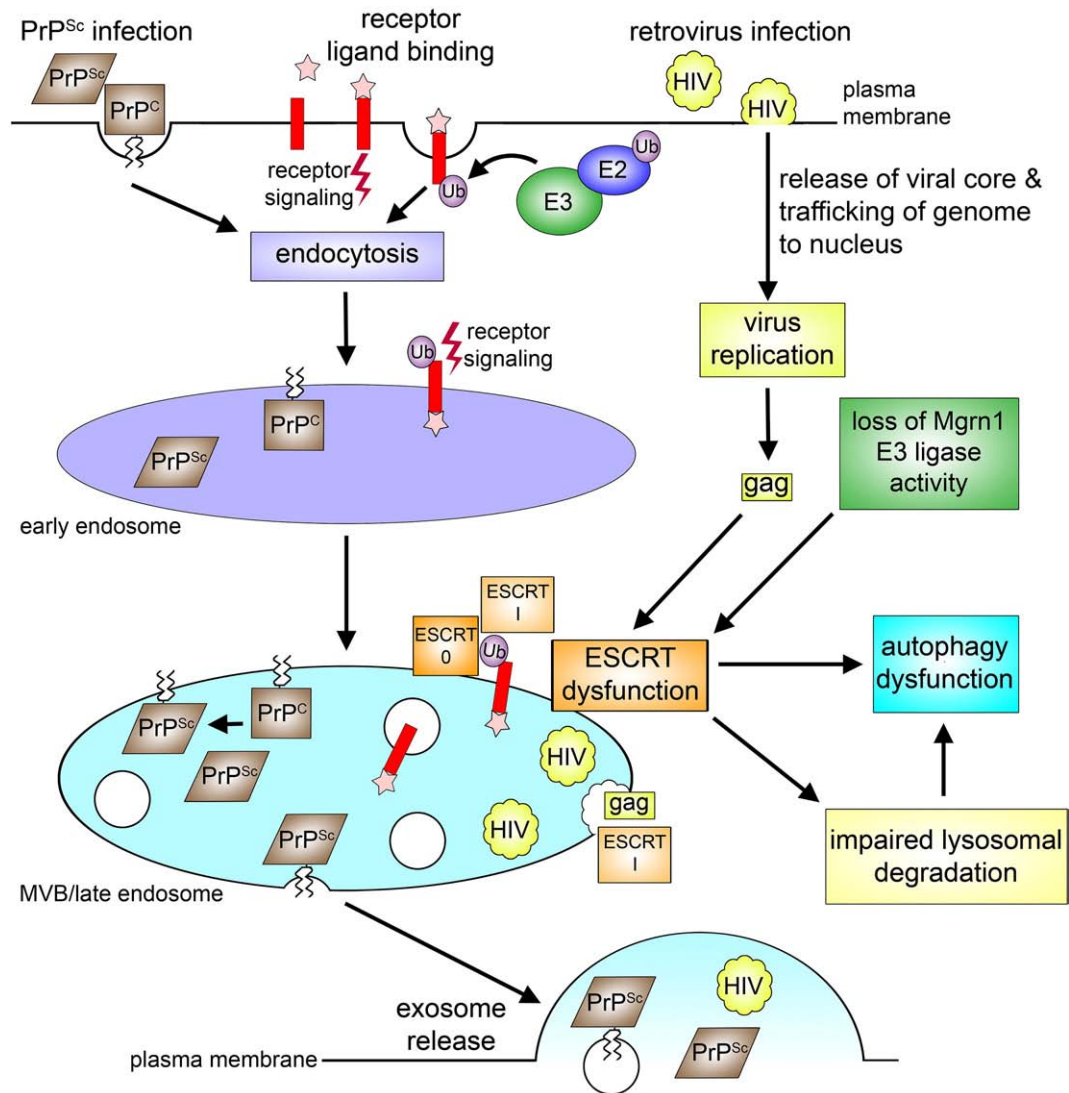


Fig. 4. Potential mechanisms by which dysfunction of ubiquitin-mediated endosome-to-lysosome trafficking in spongiform disorders results in abnormal intracellular signaling, attenuation of lysosomal protein degradation, and impairment of autophagy. Exogenous PrP^{Sc} and ligand-bound receptors enter the cell via the endocytic pathway. Receptor signaling is modulated by lysosomal degradation, which is dependent upon correct ESCRT-mediated sorting of the receptors. PrP^c trafficking is disrupted by PrP^{Sc}, which can mediate the conversion of endogenous PrP^c into PrP^{Sc} in the MVB or other endocytic compartments and accumulates in the MVB/late endosome. Infection of a cell with retroviruses results in the production of viral gag proteins, which hijack the ESCRT sorting machinery to aid in viral budding at the MVB or at the plasma membrane (not shown). Viruses and PrP in the MVB can be released from the cell via the exosome pathway. Loss of Mgrn1-mediated ubiquitination and viral gag proteins disrupt normal ESCRT sorting of cell surface receptors, which interferes with receptor degradation by the lysosome, potentiating receptor intracellular signaling. Dysfunction of both the ESCRT sorting machinery and the lysosome impair degradation of cellular proteins by autophagy.

reported for a number of spongiform disorders. Autophagy is dependent upon proper endocytic trafficking and lysosome function, which is perturbed in prion diseases, AD, and retroviral infection. We have shown that loss of the E3 ligase Mgrn1 directly impairs endocytic trafficking through its interaction with the sorting protein Tsg101, further emphasizing a role for trafficking in the pathogenesis of spongiform degeneration.

Studies of PrP trafficking and function, retrovirus replication, and Mgrn1 and Atrn function has increased our understanding of the processes involved in spongiform degeneration. Further identification of interactors of PrP, Mgrn1, Atrn, and viral proteins will help elucidate signal cascades that result in cell death and vacuolation in these disorders. Examination of different spongiform encephalopathies has illuminated several key cellular pathways that are affected in the pathogenesis of vacuolar change, which include ubiquitin signaling, protein degradation, oxidative stress, autophagy, and intracellular trafficking. More understanding of specific proteins in the cellular processes that result in spongiform pathology will enable researchers

to identify drug targets to slow down or halt the progression of spongiform pathology in patients with these disorders.

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