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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 fluffily 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...
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 ubiquitinating enzymes 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. Prior 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–bound 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...
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 [15,47].

The conformational change of normal cellular PrP protein (PrPC, cellular) into a disease-related structure (PrPSc, 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 prion animal diseases, infection with or consumption of tissues containing PrPSc mediates the propagation of PrPSc from PrPC. PrPSc is necessary for disease pathogenesis, since animals or cells that lack PrPC are resistant to PrPSc infection and fail to propagate prion infectivity [52-55]. This supports the hypothesis that PrPSc acts as a template for the misfolding of PrPC 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. PrPSc has been shown to be ubiquitinated and processed by the UPS [39], while PrPSc 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 PrPSc [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 PrPSc, 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 PrPSc 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

| Disease/Mutation | CNS location of spongiform change | Increased ubiquitination | Altered prion protein | Gliosis | Ref (s) |
|------------------|----------------------------------|--------------------------|-----------------------|---------|--------|
| Scapie 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     | [20,21,82,83] |
| Coronavirus-infected mouse | Grey matter of cortex, hippocampus, medulla, brainstem, spinal cord | ND                      | ND                    | Yes     | [8,18] |
| Mgeni '93-infected mouse | Grey matter of cortex, cerebellum, hippocampus, medulla, brainstem | ND                      | No                    | Yes     | [22]   |
| Canavan’s spongiform leukodystrophy | Grey matter of cerebellum, hypothalamus, medulla | Yes                      | Yes                   | No      | [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 PrPSc 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]. Signalizing 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<sup>+</sup>) 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<sup>+</sup> and mahogany phenotypes, biochemical interaction between Mgrn1 and Atrn has not been established. Atrn protein levels are normal in Mgrn1<sup>+</sup> 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 melano-cortin 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<sup>sc</sup> [22,117]. While PrP is ubiquitinated [39], Mgrn1 does not ubiquinate PrP [22], implying that the spongiform degeneration seen in Mgrn1<sup>+</sup> 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 in Mgrn1<sup>-</sup> mice [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<sup>-</sup> 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

The aberrant ubiquitination observed in spongiform encephalo-pathies 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 fission and fusion 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<sup>-</sup> 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 Attn in hypothalamic melanocortin signaling is unclear, although Attn 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 [139] 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 [99,156,67], 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 Vila 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 Mgrn1md-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 proteasome 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 PrPc into PrPSc. Experimental treatment of recombinant PrPc with ROS in vitro generates oxidized PrP that tends to aggregate, is protease-resistant, and is capable of templating itself, much like PrPSc [167]. Aggregation of PrPSc can inhibit the proteasome [166] and PrP aggregation can activate apoptotic pathways [170]. Oxidative stress signaling via the ERK pathway is influenced by both PrPc [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 PrPc in the cellular regulation of oxidative stress [171] and suggesting that increases in protein aggregation and decreased proteasome activity may result from loss of PrPc 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 Mgrn1md-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, increased 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 mole-}

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ules 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
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 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.

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-mono ubiquitination) 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].

PrPSc is a glycosylphosphatidylinositol (GPI)-anchored glycoprotein which 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 PrPSc missing the GPI anchor are resistant to clinical disease after infection with scrapie, similar to PrP knockout mice, despite having high levels of PrPSc and amyloid plaques in their brains [202]. This indicates PrPSc toxicity is likely dependent upon its localization to the plasma membrane and/or endocytic system. The conformational change from PrP to PrPSc 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 (PrPSc) accumulates in fractions that also contain markers for the late endosome and lysosome. This is in contrast to protease-sensitive PrP (PrPSc), which is prevalent in lighter fractions representing early endosomes [63], indicating that the conversion of PrP to PrPSc 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 PrPSc compared to PrPSc 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 PrPSc release is increased in cells that are infected with both PrPSc 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 multimonoubiquitinites Tsg101. Small interfering RNA-mediated knockdown 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 mouse 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). Division 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 PrPSc 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, Nf2, 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
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, including 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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