Chapter

A Molecular Mechanism for Abnormal Prion Protein Accumulation

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Abstract

A fundamental event in the pathogenesis of prion disease is the conversion of cellular prion protein into an abnormally folded isoform (PrP^{Sc}), which is the infectious causative agent of disease. With progression of disease, PrP^{Sc} is replicated and excessively accumulated in most cases. However, the molecular mechanism for excessive accumulation of PrP^{Sc} is not well understood. Recently, Sortilin, a member of the VPS10P domain receptor family, has been identified as a sorting receptor that directs prion protein (PrP) to the lysosomal degradation pathway. Moreover, it has been shown that prion infection impairs Sortilin function, resulting in delayed PrP^{Sc} degradation. In this chapter, we explain the mechanisms for PrP trafficking into the lysosomal degradation pathway mediated by Sortilin and overaccumulation of PrP^{Sc} caused by Sortilin dysfunction.

Keywords: PrP^{Sc}, PrP^{Sc} accumulation, PrP^{Sc} degradation, Sortilin, sorting, VPS10P domain, sorting receptor, VPS10P domain receptor

1. Introduction

Prion diseases are a group of fatal neurodegenerative disorders that are caused by the transmissible misfolded isoform (PrP^{Sc}) of the cellular prion (PrP^{C}) [1], including Creutzfeldt-Jakob disease of humans, bovine spongiform encephalopathy, and scrapie of sheep. PrP^{Sc} is a β-sheet rich conformer of PrP^{C} and is partially resistant to protease. With progression of prion disease, PrP^{Sc} is replicated and accumulated in the brain, and neuronal dysfunction and death occur. Previous studies have shown that PrP-null mice neither develop the disease nor accumulate PrP^{Sc} even after prions are inoculated into their brains [2, 3]. This indicates that replication and accumulation of PrP^{Sc} are closely related to the pathogenesis of prion disease. Therefore, elucidation of the mechanisms of PrP^{Sc} degradation and accumulation is critical for understanding the pathogenic mechanism of prion disease and for developing therapeutic agents.

PrP^{Sc} usually accumulates excessively over PrP^{C} in cultured cells and mouse brains (**Figure 1**). This strongly indicates that PrP^{Sc} is protected against its proteolytic degradation. Actually, several studies have reported that the proteolytic systems (e.g., lysosomal degradation and ubiquitin-proteasomal degradation systems) are inhibited by prion infection [4–7], and PrP^{Sc} is found at the cell surface and in endosomal/lysosomal compartments [8–10]. Moreover, when PrP^{Sc} was

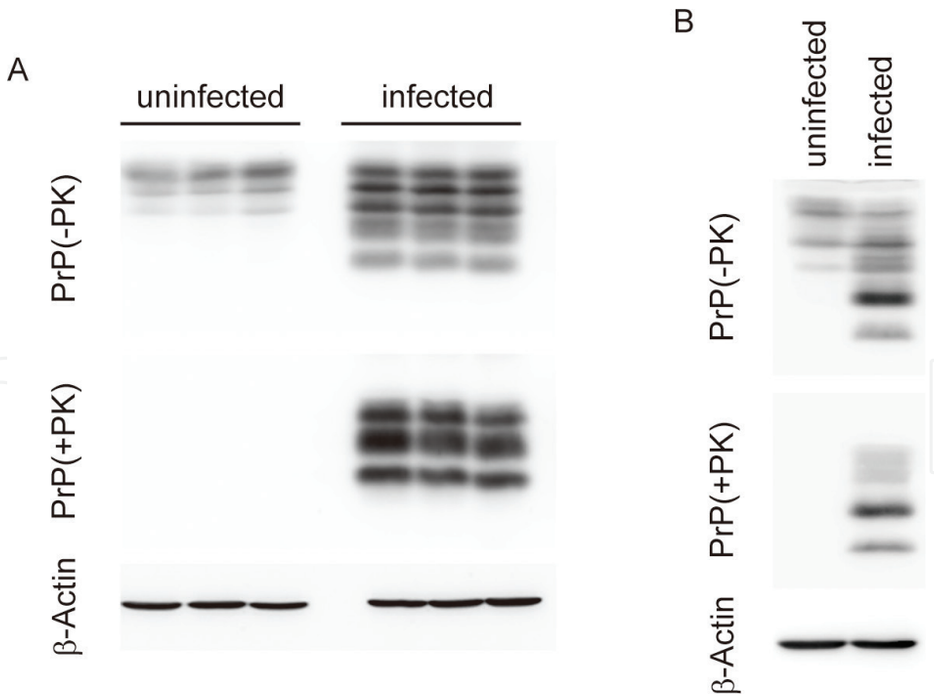

Figure 1.
PrP expression in mice brain and N2a cells. (A) Total PrP and PrP^{Sc} were compared between RML prion infected mouse brains at terminal stage and age matched uninfected mice brain by western blotting. (B) N2a cells were treated with uninfected or 22 L-prion infected mice brain homogenate. At 30 dpi, total PrP and PrP^{Sc} were detected by western blotting. Blots were probed with anti-PrP antibody (6D11) and anti-β-actin antibody.

fractionated by detergent-based biochemical fractionation, most of the PrP^{Sc} was detected in detergent-resistant membrane (DRM) fractions [11], suggesting that PrP^{Sc} mainly exists in membrane bound form and PrP^{Sc} is degraded preferentially in lysosomes, but not by cytosolic proteasomes. PrP^{Sc} to be degraded in lysosomes might be preferentially selected and directed into the lysosomal degradation pathway by dedicated membrane trafficking machinery. Therefore, knowledge of the mechanism that sorts PrP into late endosomal/lysosomal compartments should be important for understanding the accumulation of PrP^{Sc}.

2. PrP^{Sc} accumulation

Figure 1A shows the expression of total PrP and PrP^{Sc} in uninfected and prion-infected mouse brains. In this figure, we can easily recognize that the total amount of PrP in infected mouse brains is larger than in uninfected mouse brains. In cultured cells, such excessive expression of total PrP in infected cells was also confirmed (**Figure 1B**). These results indicate that the amount of PrP^{Sc} in infected cells is larger than PrP^{C} in uninfected cells, and that PrP^{Sc} is protected against proteolytic degradation.

Why is PrP^{Sc} protected from proteolysis and over-accumulated? One possible reason is the protease resistance of PrP^{Sc} that is attributed to its β-rich structure at the C-terminal region. If such protease resistance mainly affected the inhibition of PrP^{Sc} degradation, most of the PrP^{Sc} could be found in the lysosome, which contains various kinds of hydrolytic enzymes and is a major compartment responsible for the digestion of macromolecules such as proteins. The majority of PrP^{Sc} is actually observed intracellularly, whereas PrP^{C} mainly localizes to the cell surface (**Figure 2A**). However, detailed analyses of its intracellular distribution show that PrP^{Sc} is widely distributed in posttrans Golgi network (TGN) compartments [8–10] (**Figure 2B**). From these

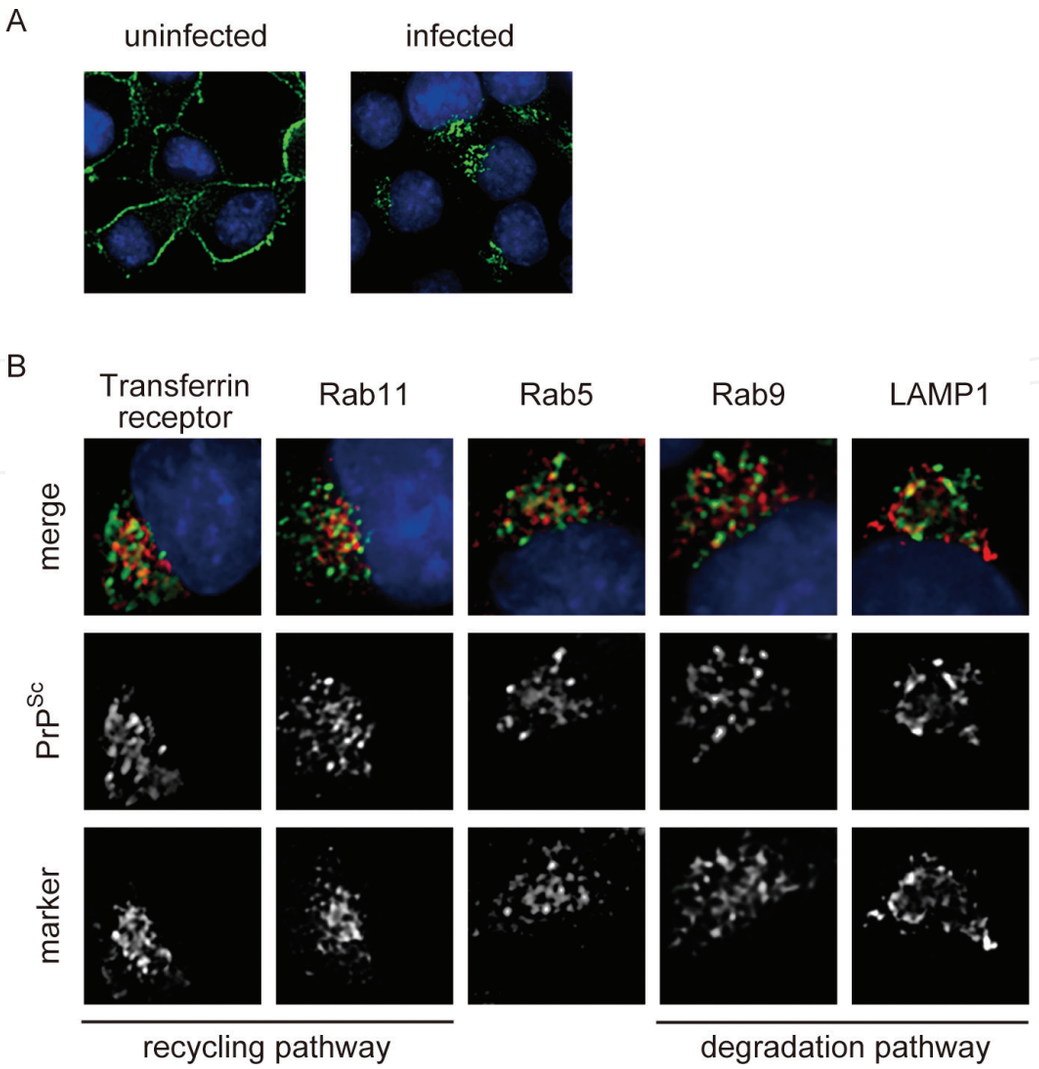

Figure 2.
PrP^{Sc} is widely distributed in post-Golgi compartments. (A) PrP^{C} (green, uninfected cells) and PrP^{Sc} (green, infected cells) were visualized by immunofluorescence staining with mouse monoclonal anti-PrP antibody (SAF83) and anti-PrP^{Sc} antibody (132), respectively. (B) PrP^{Sc} indicated organelle markers in prion infected cells were doubly stained with anti-PrP^{Sc} antibody (132) and anti-transferrin receptor, Rab11, Rab5, Rab9 and LAMP1 antibody, respectively. DAPI was used for nuclear stain (blue).

observations, it seems that impairment of PrP^{Sc} trafficking into lysosomes as well as its protease-resistance causes inhibition of degradation and over-accumulation of PrP^{Sc}.

3. Sortilin and other VPS10P domain receptors

PrP would have to move by transport vesicles in post-TGN compartments, including TGN, endosomes, lysosomes, and the plasma membrane. Then, in this transport network, the PrP to be degraded could be sorted into transport carriers bound for late endosomal/lysosomal compartments. For this purpose, a sorting receptor might be useful and required because it can select and concentrate a target cargo protein into transport carriers and promote transport carrier formation. In our recent study, Sortilin has been identified as a sorting receptor that directs PrP into late endosomal/lysosomal compartments. Sortilin is a member of the VPS10P domain receptor family, which is comprised of five members (Sortilin, SorCS1, SorCS2, SorCS3, and SorLA). In this section, briefly, we describe Sortilin and other VPS10P receptors and their implications for neurodegenerative diseases.

VPS10P-domain receptors are multiligand type-I transmembrane proteins. They contain five members, Sortilin, SorLA, SorCS1, SorCS2, and SorCS3, and deliver a number of target cargo proteins to their destinations, interacting with them via VPS10P domains on the luminal/extracellular N-terminus (**Figure 3**). The whole luminal/extracellular region in Sortilin is composed of a simple VPS10P domain, but other receptors have additional modules (**Figure 3**).

VPS10P-domain receptors are expressed in the brain and are involved in neuronal function and viability [12, 13]. Sortilin binds to progranulin and mediates endocytosis and delivery of progranulin into lysosomes [14], and rare nonsynonymous variants in SORT1 increase the risk for frontotemporal lobar degeneration [15]. Sortilin also mediates trafficking of neuronal degeneration causative and related proteins. Sortilin has been identified as an amyloid precursor protein (APP) interaction partner and promotes α-cleavage of APP [16]. In addition, Sortilin interacts with BACE1, β-site APP cleavage enzyme 1, and mediates its retrograde trafficking from the plasma membrane to TGN via early endosomes [17]. It has been suggested that Sortilin is potentially associated with Parkinson's disease [18]. Moreover, recently, it has been reported that Sortilin is involved in tau prion replication [19].

As for other VPS10P receptors, it has been reported that SorLA is associated with sporadic and late-onset Alzheimer's disease (AD) [5, 20]. SorLA directs APP into the recycling pathway and protects APP from β-cleavage resulting in Aβ generation [5, 21, 22]. On the other hand, loss of SorLA shifts the traffic flow of APP to the late endosomal pathway and facilitates β-cleavage of APP and Aβ-generation [5, 21, 22]. In addition, a meta-analysis indicated that multiple SorLA variants are associated with the risk of Alzheimer's disease [23]. SorCS1 is also involved in APP transport and Aβ-generation and is identified as a risk factor for Alzheimer's disease [24, 25]. Variants of SorCS2 and SorCS3 are also associated with the risk of Alzheimer's disease [24, 25]. Although a number of studies have indicated that VPS10P-domain receptors are

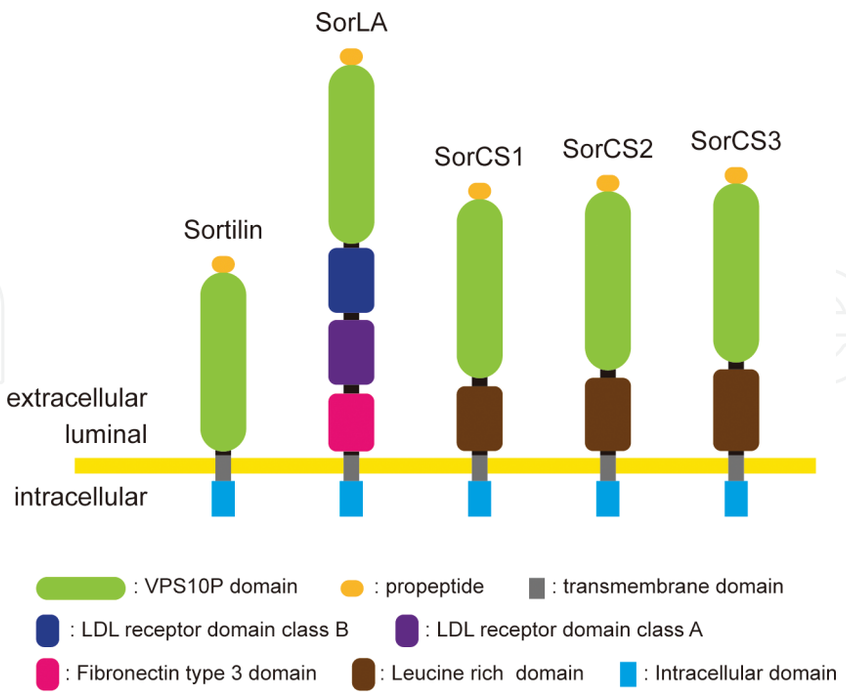

Figure 3.
VPS10P domain receptors. VPS10P-domain receptors are multiligand type-I transmembrane proteins. They contain five members, Sortilin, SorLA, SorCS1, SorCS2 and SorCS3. The extracellular/luminal region of VPS10P receptors contains VPS10P domain and additional domains. The intracellular domain of VPS10P receptors contains motifs for interaction with adaptor proteins. The propeptide at N-terminal region is cleaved by furin in the TGN.

implicated in neurodegenerative diseases and their impairment could be a risk factor for diseases, the relation between VPS10P receptors and prion disease is not known.

4. Role of Sortilin in PrP trafficking

Sortilin has been identified as a novel PrP-binding protein and is colocalized with PrP^C both at the cell surface and intracellular compartments [11]. In Sortilin-knockdown (Sortilin-KD) uninfected cells, most of the PrP^C is localized at the cell surface, and PrP^C expression is increased. In addition, a PrP^C uptake experiment, in which cell surface PrP^C was labeled with anti-PrP antibody and internalized labeled PrP^C was measured after incubation, demonstrated that PrP^C internalization was weakened by Sortilin-KD [11]. These results indicate that Sortilin acts as a cell surface receptor for PrP^C endocytosis.

PrP^C was also colocalized with Sortilin intracellularly [11]. This made us recollect that Sortilin could function intracellularly as a sorting receptor for PrP trafficking. When the internalized labeled PrP^C was costained for either Rab9 (a late endosomal marker) or Rab11 (a recycling endosomal marker) by indirect immunofluorescence, the internalized PrP^C distributed to both late and recycling endosomes in control cells, whereas, in Sortilin-depleted cells, it failed to localize to late endosomes, and most of the internalized PrP^C is localized to recycling endosomes [11]. These observations indicate that Sortilin is also required for sorting of PrP^C into late endosomes to degrade it.

Moreover, when wild type (wt) and Sortilin-knockout (ΔSort) cells were treated with NH_4Cl, which increases lysosomal pH and inhibits proteolytic enzymes in lysosomes, PrP^C was effectively accumulated in wt but not in ΔSort cells [11], and PrP^C colocalization with LAMP1, a lysosomal marker, in NH_4Cl-treated ΔSort cells was significantly lower than NH_4Cl-treated wt cells [11]. These results suggest that ΔSort cells failed to transport PrP^C properly into lysosomes.

Altogether, it could be concluded that Sortilin functions as a cell surface receptor for PrP^C internalization and a sorting receptor to direct PrP^C to lysosomes via late endosomes (**Figure 4**). We would be able to extend such a role of Sortilin in PrP^C trafficking to PrP^{Sc} because Sortilin directly interacted with PrP^C through its highly flexible N-terminal domain and anti-Sortilin antibody coprecipitated both PrP^C and PrP^{Sc}. In practical terms, Sortilin is implicated in PrP^{Sc} degradation.

The inhibition of Sortilin inhibited PrP^C internalization by ~20% in the PrP^C uptake assay [11]. This result raises a question. Why is PrP^C endocytosis inhibited partially even when Sortilin function is almost or completely abolished [11]? There are suggestive findings to answer this question. We examined the PrP distribution in uninfected wt cells and in uninfected ΔSort cells by detergent-based biochemical fractionation. Sixty three percent of PrP^C in wt cells was detected in detergent resistant membrane (DRM) fractions, generally recognized as raft fractions, but thirty-seven percent of PrP^C was also found in detergent soluble (nonraft) fractions [11]. Sortilin deficiency changed the PrP^C distribution, and PrP^C in nonraft fractions was reduced to ~15% in ΔSort cells [11]. At present, it is thought that both lipid raft- and clathrin-mediated endocytosis execute PrP^C internalization [13, 26]. Sortilin was mostly isolated in nonraft fractions [11]. It has been reported that the cytoplasmic tail of Sortilin can interact with clathrin-associated adaptor protein complex, AP-2, at the plasma membrane and facilitate clathrin-mediated endocytosis [13, 27, 28]. We showed that the recombinant PrP devoid of its N-terminal domain (residues 23–88) (PrPΔ23–88) did not bind to Sortilin. Additionally, internalization and lysosomal degradation of PrPΔ23–88 were inhibited, and it accumulated at the cell surface [11]. These results are in good agreement with a previous report: the

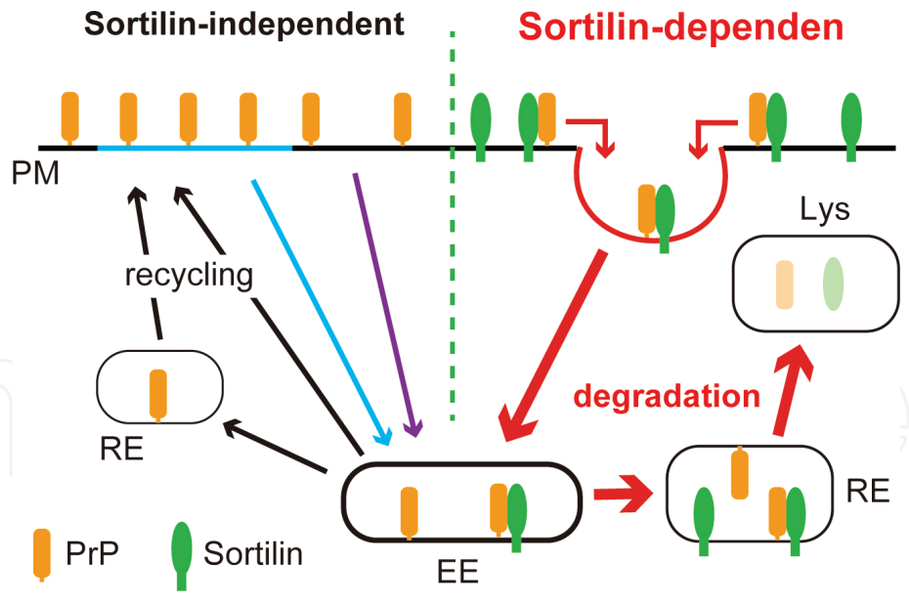

Figure 4.
Role of Sortilin in PrP-trafficking. Sortilin internalizes PrP from nonraft domain and direct into late endosomal/lysosomal degradation pathway. PrP internalized from lipid raft domain in Sortilin-independent manner would be largely recycled into cell surface. PrP might be also internalized from nonraft domain in Sortilin-independent manner. Red arrows indicate Sortilin mediated PrP-trafficking pathway. Blue line is lipid raft domain. EE: Early endosome, LE: Late endosomes, RE: Recycling endosomes, Lys: Lysosomes, PM: Plasma membrane.

N-terminal domain (residues 23–107) of PrP^{C} is sufficient for its endocytosis mediated by clathrin [29]. It is therefore inferred that Sortilin internalizes PrP^{C} from nonraft domains at the cell surface by clathrin-coated vesicles. Moreover, it has been shown that the expression of total PrP^{C} was not changed even when the flotillin-1–mediated lipid raft-dependent endocytosis of PrP^{C} was inhibited by the knockdown of flotillin-1 [30]. Their and our results suggest that Sortilin-mediated endocytosis directs PrP^{C} into the late endosomal/lysosomal degradation pathway, whereas PrP^{C} that is internalized from the lipid raft domain in a Sortilin-independent manner largely enters the recycling pathway (**Figure 4**).

5. Dysfunction of Sortilin by prion infection

Sortilin expression also affects PrP^{Sc} levels. Sortilin-KD increased PrP^{Sc} in prion infected cells, similarly to PrP^{C} in uninfected cells [11]. On the contrary, overexpression of Sortilin in infected cells reduced PrP^{Sc} [11]. Furthermore, when we investigated PrP^{Sc} accumulation in $Sort1^{+/+}$ and $Sort1^{-/-}$ mouse brains after intracerebral prion inoculation, PrP^{Sc} levels in $Sort1^{-/-}$ mouse brains were significantly higher than in $Sort1^{+/+}$ mouse brains at the early stages of disease (at 45, 60, 90 dpi) [11], suggesting an inhibition of PrP^{Sc} degradation. Namely, dysfunction of Sortilin causes excessive accumulation. If so, does prion infection inhibit Sortilin function? Notably, Sortilin in infected cells was ~50% lower than in uninfected cells [11]. Moreover, in infected mouse brains at terminal stage, Sortilin also fell to ~45% as compared with age-matched uninfected mice [11]. These observations suggested that prion infection downregulated Sortilin expression. To confirm this, uninfected cells were treated with RML prion-infected mouse brain homogenate, and Sortilin and PrP^{Sc} in individual cells were visualized by double immunofluorescence staining at 9 dpi (**Figure 5**). In cells displaying bright green signals derived from PrP^{Sc}, little Sortilin (red) was detected, whereas the bright red fluorescence of Sortilin was observed in the others; that is, Sortilin expression was reduced by prion infection.

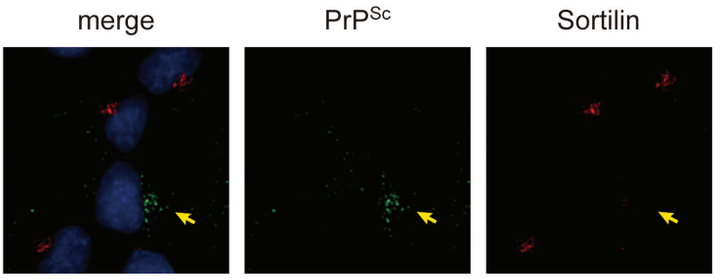

Figure 5.
Prion infection reduces Sortilin expression. Immunofluorescence staining of Sortilin (red) and PrP^{Sc} (green) 9 days after infection of uninfected cells with RML prions. Four horizontal serial images at 1 μm interval were collected, and orthogonally projected image was created. DAPI was used for nuclear stain (blue). Yellow arrow indicates PrP^{Sc}-positive cell.

To clarify why Sortilin is reduced by prion infection, we examined mRNA transcript levels by RT-PCR. There was little difference in Sortilin mRNA abundance between uninfected and infected cells. This suggested that the degradation of Sortilin was facilitated in prion infected cells. Hence, we treated cells with inhibitors of proteolytic degradation. The expression of Sortilin was almost the same in both untreated and MG132-treated cells but increased in NH_4Cl-treated cells [11]. In particular, Sortilin expression was dramatically improved in NH_4Cl-treated prion-infected cells, and another lysosomal inhibitor, concanamycin A, also improved Sortilin expression in infected cells [11], suggesting that Sortilin is over-degraded in prion-infected cells in lysosomes.

6. Conclusions

Sortilin has been identified as a novel PrP-binding protein and functions as a sorting receptor to direct PrP into late endosomal/lysosomal compartments.

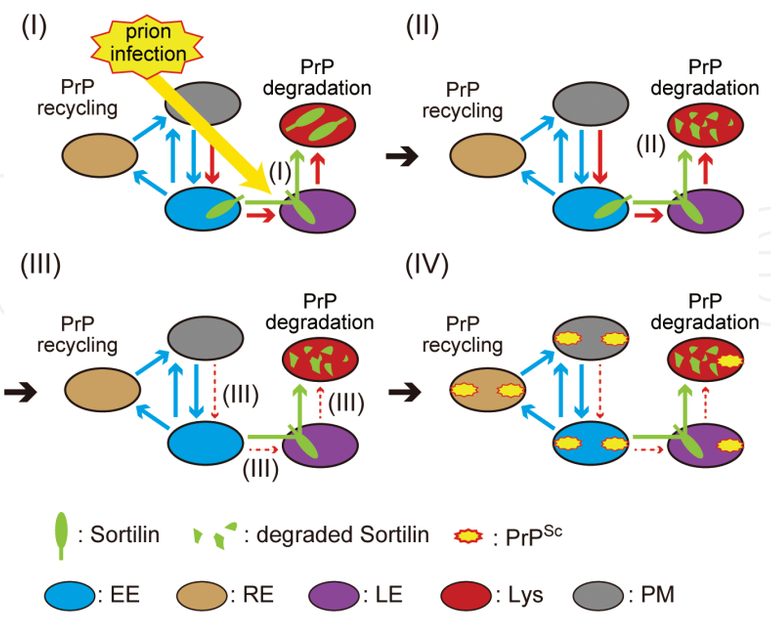

Figure 6.
Possible mechanism for PrP^{Sc} over-accumulation by prion infection. (I) the entry of Sortilin into the lysosomal degradation pathway is facilitated (green arrow) by prion infection (yellow arrow), (II) Sortilin is over-degraded in lysosomes, (III) trafficking of PrP^{Sc} to late endosomal/lysosomal compartments is restricted (red broken arrow), and (IV) PrP^{Sc} is protected against its degradation in lysosomes and is excessively accumulated. Red arrows indicate Sortilin-mediated PrP-trafficking pathway and blue arrows indicate other PrP-trafficking pathways. EE: Early endosomes, LE: Late endosomes, RE: Recycling endosomes, Lys: Lysosomes, PM: Plasma membrane.

Dysfunction of Sortilin induces delayed degradation and excessive accumulation of PrP. Notably, prion infection downregulated Sortilin expression by facilitating Sortilin degradation in lysosomes. Finally, we summarize a possible mechanism of excessive accumulation of PrP^{Sc} during prion infection (**Figure 6**): (I) the entry of Sortilin into the lysosomal degradation pathway is facilitated by prion infection, (II) Sortilin is over-degraded in lysosomes, (III) trafficking of PrP^{Sc} to late endosomal/lysosomal compartments is restricted, and (IV) PrP^{Sc} is protected against its degradation in lysosomes and is excessively accumulated. However, it still remains unclear how prion infection facilitates Sortilin degradation in lysosomes.

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Conflict of interest

The authors have declared that no competing interests exist.

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