Novel Amplification Mechanism of Prions through Disrupting Sortilin-Mediated Trafficking

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Short title: Prion accumulation via sortilin dysfunction

ABSTRACT.

Conformational conversion of the cellular prion protein, PrP^{C}, into the abnormally folded isoform of prion protein, PrP^{Sc}, which leads to marked accumulation of PrP^{Sc} in brains, is a key pathogenic event in prion diseases, a group of fatal neurodegenerative disorders caused by prions. However, the exact mechanism of PrP^{Sc} accumulation in prion-infected neurons remains unknown. We recently reported a novel cellular mechanism to support PrP^{Sc} accumulation in prion-infected neurons, in which PrP^{Sc} itself promotes its accumulation by evading the cellular inhibitory mechanism, which is newly identified in our recent study. We showed that the VPS10P sorting receptor sortilin negatively regulates PrP^{Sc} accumulation in prion-infected neurons, by interacting with PrP^{C} and PrP^{Sc} and trafficking them to lysosomes for degradation. However, PrP^{Sc} stimulated lysosomal degradation of sortilin, disrupting the sortilin-mediated degradation of PrP^{C} and PrP^{Sc} and eventually evoking further accumulation of PrP^{Sc} in prion-infected neurons. These findings suggest a positive feedback amplification mechanism for PrP^{Sc} accumulation in prion-infected neurons.

KEYWORDS. Prions, prion protein, sortilin, sorting, VPS10P sorting receptor, protein degradation, lysosome.

Introduction

Prions are causative agents of prion diseases, a group of fatal neurodegenerative disorders including Creutzfeldt-Jakob disease in humans and bovine spongiform encephalopathy and scrapie in animals.[1] They are widely believed to consist of the abnormally folded, amyloidogenic isoform of prion protein, designated PrP^{Sc}.[1] PrP^{Sc} is produced through conformational conversion of the cellular prion protein, PrP^{C}, by unknown mechanisms.[1] PrP^{C} is a glycosylphosphatidylinositol (GPI)-anchored membrane glycoprotein expressed most abundantly in brains, particularly by neurons.[2] The constitutive conversion of PrP^{C} into PrP^{Sc} leads to accumulation of PrP^{Sc} in brains. We and others have shown that the conversion of PrP^{C} into PrP^{Sc} is a key pathogenic event in prion disease, by demonstrating that mice devoid of PrP^{C} neither developed the disease nor propagated prions or accumulated PrP^{Sc} in their brains after intracerebral inoculation with prions.[3-6] Most pathogens usually evade host defense mechanisms to propagate themselves in their hosts. However, the host defense mechanism against prions to suppress prion propagation, or PrP^{Sc} accumulation, remains unknown.

The vacuolar protein sorting-10 protein (VPS10P)-domain receptors, including sortilin, SorLA, SorCS1, SorCS2 and SorCS3, are multi-ligand type I transmembrane proteins abundantly expressed in brains and involved in neuronal function and viability.[7, 8] They function as a cargo receptor to deliver a number of cargo proteins to their subcellular compartments through the VPS10P domain in the extracellular luminal N-terminus.[7, 8] Accumulating lines of evidence indicate that altered VPS10P receptor-mediated trafficking

could be involved in the pathogenesis of neurodegenerative disorders, including Alzheimer's disease[9-12] and frontotemporal lobar degeneration.[13] Sortilin mediates intracellular trafficking of the amyloid precursor protein (APP)-cleaving enzyme BACE1[14] and the neurotrophic factor receptors Trks.[15] SorLA and SorCS1 are involved in APP transport.[9, 11]

We recently reported that sortilin negatively regulates PrP^{Sc} accumulation by sorting PrP^{C} and PrP^{Sc} to lysosomes for degradation, and that PrP^{Sc} accumulation itself impairs the sotrtilin-mediated degradation of PrP^{C} and PrP^{Sc} by stimulating lysosomal degradation of sortilin, thereby evoking further accumulation of PrP^{Sc} in prion-infected cells.[16] These findings suggest that the sortilin-mediated lysosomal degradation of PrP^{C} and PrP^{Sc} could be a host defense mechanism against prions, and that prions, or PrP^{Sc}, could propagate in infected neurons by evading the sortilin-mediated defense mechanism by inducing lysosomal degradation of sortilin.

Sortilin is a negative regulator for PrP^{Sc} accumulation

We found that PrP^{C} directly interacts with sortilin, but not with other VPS10P molecules, on the plasma membrane in PrP^{C}-overexpressing neuroblastoma N2a cells, designated N2aC24 cells.[16] The interaction of both molecules was also confirmed in mouse brain homogenates.[16] SiRNA-mediated knockdown of sortilin increased PrP^{Sc} in prion-infected N2aC24L1-3 cells, which are N2aC24 cells persistently infected with 22L scrapie prions.[16] In contrast, overexpression of sortilin in N2aC24L1-3 cells decreased PrP^{Sc}.[16] We also

showed that sortilin-knockout mice had accelerated prion disease caused by early accumulation of PrP^{Sc} in their brains after infection with RML scrapie prions.[16] These results indicate that sortilin could negatively regulate PrP^{Sc} accumulation in prion-infected cells and mice.

Sortilin traffics PrP^{C} to non-raft domains and to late endosomes/lysosomes

PrP^{C} is synthesized in the endoplasmic reticulum (ER) and trafficked to the plasma membrane through the Golgi apparatus.[17] PrP^{C} undergoes several posttranslational modifications during its biosynthesis, including cleavage of the N-terminal signal peptide, removal of the C-terminal peptide for attachment of a GPI anchor at the C-terminus and formation of a disulfide bond at the C-terminal domain in the ER, and addition of two core N-linked oligosaccharides at the C-terminal domain in the ER that are further modified in the ER and then in the Golgi apparatus.[17] Like other GPI-anchored proteins, PrP^{C} is predominantly located at raft domains and, to a lesser extent, at non-raft domains.[16, 17] After internalization, some PrP^{C} molecules are delivered back to the plasma membrane directly or indirectly via the recycling endosome compartments and others are transported to lysosomes for degradation.[18] Copper and zinc stimulate endocytosis of PrP^{C} by binding to histidine residues in the octapeptide repeat (OR) region located in the N-terminal domain.[19-21] It has been postulated that PrP^{C} interacts with an as yet unidentified raft molecule via the N-terminal domain including the OR region, thereby being retained at raft domains.[20] The binding of copper or zinc to the OR region causes structural changes in the

N-terminal interacting region of PrP^C, thereby PrP^C leaves raft domains to non-raft domains to be endocytosed via the clathrin-dependent pathway.[20] Low-density lipoprotein receptor-related protein 1 has been reported to be involved in the clathrin-dependent endocytosis of PrP^C.[22] The clathrin-independent pathways including caveolae, which is considered to be formed by clustering raft domains, or caveolae-like domains have been also reported to mediate the endocytosis of PrP^C.[18]

We found that sortilin was predominantly located at non-raft domains in prion-uninfected N2aC24 cells.[16] Sortilin knockout caused marked shift in localization of PrP^C from non-raft domains to raft domains in N2aC24 cells and mouse brains.[16] These findings suggest that sortilin could function to recruit PrP^C from raft domains to non-raft domains. We also found that, after internalization, PrP^C was transported to both late endosomes and recycling endosomes in N2aC24 cells.[16] However, PrP^C was preferentially transported to recycling endosomes with reduced localization at late endosomes/lysosomes in sortilin-knockdown and -knockout N2aC24 cells,[16] indicating that sortilin also could function as an endocytic receptor for PrP^C at non-raft domains to be sent to lysosomes for degradation (Fig. 1A). Consistent with this, sortilin-deficient N2aC24 cells showed higher PrP^C on their plasma membranes than control N2aC24 cells.[16] Sortilin-knockout mice also showed higher PrP^C in their brains compared to WT mice.[16] Moreover, inhibition of lysosomal enzymes by NH_4Cl increased PrP^C markedly in N2aC24 cells, but only slightly in sortilin-knockout N2aC24 cells.[16]

The plasma membrane or raft domains are considered to be major sites for the

conversion of PrP^{C} into PrP^{Sc},[23] although the exact site of PrP^{Sc} production remains controversial. It is thus likely that sortilin could negatively regulate PrP^{Sc} accumulation by reducing PrP^{C} on the plasma membrane, particularly at raft domains through recruiting PrP^{C} to non-raft domains from raft domains and sorting it to the late endosome/lysosome protein degradation pathway.

Sortilin is involved in degradation of PrP^{Sc}

We also found that sortilin could function to direct PrP^{Sc} for degradation.[16] Sortilin interacted with PrP^{Sc} in prion-infected N2aC24L1-3 cells.[16] Sortilin-knockout significantly slowed down the degradation of PrP^{Sc} in N2aC24 cells infected with RML or 22L prions.[16] PrP^{Sc} is found at various intracellular compartments, including the plasma membrane, various endosomal compartments such as early and late endosomes, recycling endosomes, and lysosomes, and the Golgi apparatus.[18] Enzymatic release of PrP^{C} from the plasma membrane by phosphoinositide-specific phospholipase C was shown to reduce PrP^{Sc} in infected cells,[24] and formation of PrP^{Sc} was inhibited by lowered temperature,[25] which blocks the endocytosis and internalization of PrP^{C}. These suggest that the conversion of PrP^{C} into PrP^{Sc} might occur at the plasma membrane, where exogenous PrP^{Sc} is likely to first contact endogenous PrP^{C}, or after its internalization in the endosomal compartment. Internalized PrP^{Sc} could also undergo retrograde transport to the Golgi apparatus and/or to the ER,[26, 27] where the transported PrP^{Sc} might trigger the conversion of PrP^{C} into PrP^{Sc}. PrP^{Sc} molecules on the plasma membrane are trafficked to lysosomes for degradation via

the endolysosomal pathway.[28, 29] The PrP^{Sc} retrogradely transported to the Golgi apparatus are subjected to Golgi quality control and trafficked to lysosomes for degradation.[27] Sortilin localizes in para-nuclear vesicles, in the trans-Golgi network, and on the plasma membrane.[30, 31] It is thus possible that sortilin could be involved in both degradation trafficking pathways of PrP^{Sc}. However, sortilin and PrP^{Sc} molecules differed in their microdomain localization on the plasma membrane in N2aC24L1-3 cells. Sortilin was predominantly detected in non-raft fractions while PrP^{Sc} was exclusively located in raft fractions (Fig. 1B).[16] Therefore, the sortilin-mediated lysosomal degradation of PrP^{Sc} located on the plasma membrane might be a minor event.

PrP^{Sc} stimulates degradation of sortilin in lysosomes

Interestingly, we found that sortilin was markedly reduced in both prion-infected cells and mouse brains, and that the reduced sortilin levels in prion-infected cells were recovered by treatment with lysosomal inhibitors but not with proteasomal inhibitor.[16] These findings suggest that sortilin is increasingly degraded in lysosomes in prion-infected cells. We also found that PrP^{Sc} accumulation preceded the reduction of sortilin in N2aC24 cells freshly infected with RML prions.[16] Immunofluorescent staining showed that sortilin was barely detectable in PrP^{Sc}-positive cells but still abundantly observed in PrP^{Sc}-negative cells.[16] It is thus likely that PrP^{Sc} produced after prion infection could stimulate sortilin degradation in lysosomes in a cell-autonomous fashion, and that the negative role of sortilin in PrP^{Sc} accumulation could be impaired in prion-infected cells, therefore PrP^{Sc} progressively

accumulates in prion-infected neurons.

Conclusions

We presented a novel accumulation mechanism of PrP^{Sc} through degradation of sortilin. Sortilin could form the host defense mechanism against prions, by functioning to sort PrP^{C} and PrP^{Sc} to the late endosomal/lysosomal compartments for degradation (Fig. 1A, B). Conversely, PrP^{Sc} itself stimulates degradation of sortilin in lysosomes, reducing sortilin levels and impairing its defense function against prions. As a result, PrP^{C} is increasingly converted to PrP^{Sc}, and PrP^{Sc} degradation is delayed, and eventually PrP^{Sc} progressively accumulates in prion-infected cells (Fig. 1B). Accelerating the sortilin-mediated lysosomal degradation of PrP^{C} and PrP^{Sc} might be beneficial for treatment of prion diseases.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors declare no competing interests.

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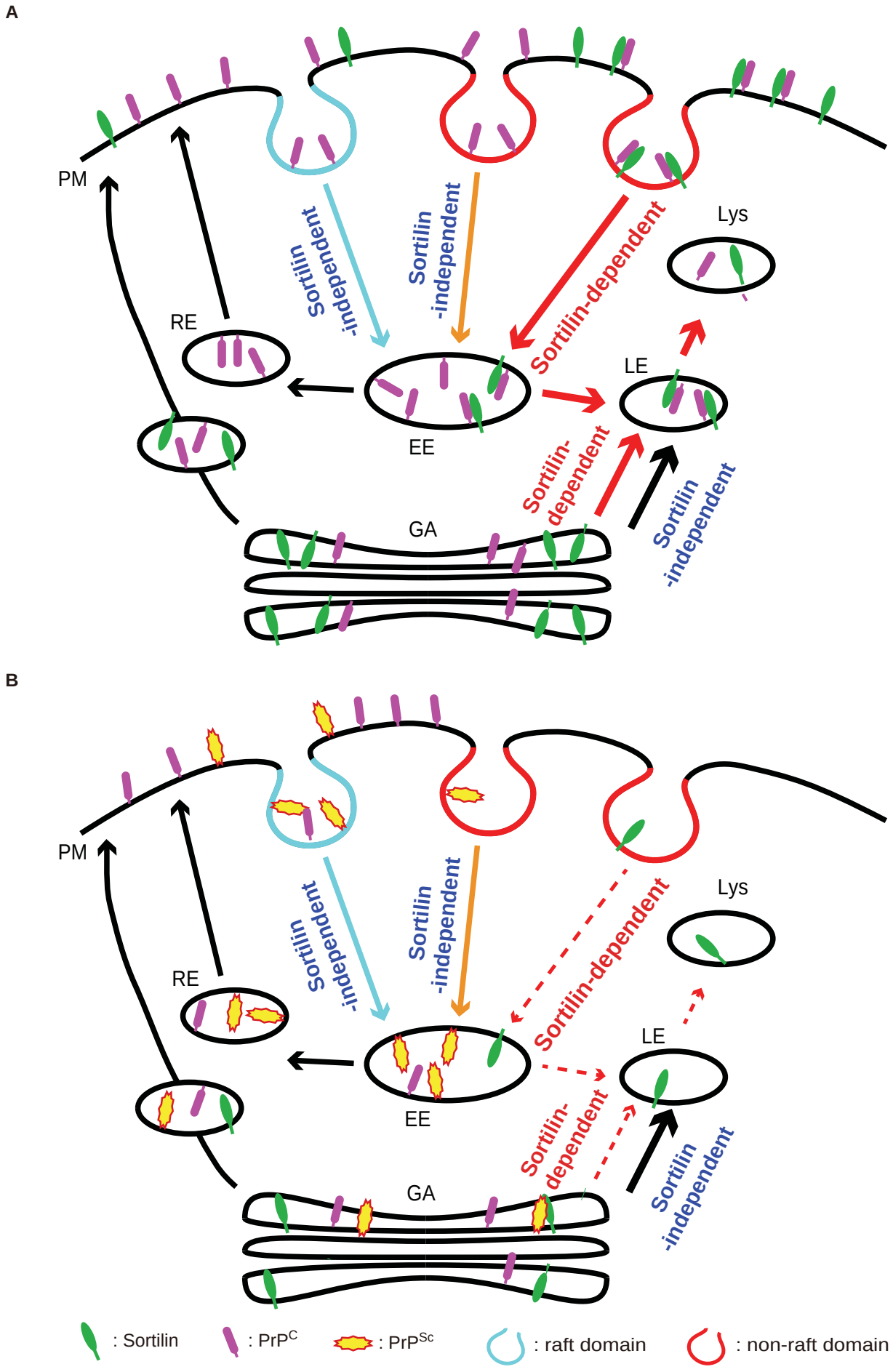
Figure Legends

Figure 1. A model of the sortilin-mediated intracellular trafficking of PrP^{C} and PrP^{Sc} in prion-uninfected and infected neurons. (A) Sortilin-dependent and -independent endocytosis of PrP^{C} in uninfected neurons. Sortilin mediates endocytosis of PrP^{C} on the plasma membrane (PM), particularly at non-raft domains, via the clathrin-dependent pathway to early endosomes (EE) and then traffics it to late endosomes/lysosomes (LE/Lys) for degradation. Other PrP^{C} molecules are trafficked either to LE/Lys for degradation or to the recycling endosome (RE) pathway in a sortilin-independent way. There also might be sortilin-dependent and -independent trafficking pathways from the Golgi apparatus (GA) to LE/Lys for degradation. (B) Intracellular trafficking of PrP^{C} and PrP^{Sc} in prion-infected neurons. Prion infection stimulates lysosomal degradation of sortilin via an unknown mechanism, thereby impairing the sortilin-mediated trafficking of PrP^{C} and PrP^{Sc} to LE/Lys for degradation. As a result, PrP^{C} and PrP^{Sc} are increased at raft domains and endocytosed via the sortilin-independent pathway to RE, causing accumulation of PrP^{Sc} and increasing conversion of PrP^{C} into PrP^{Sc} in prion-infected neurons. PrP^{Sc} could undergo retrograde transport to the GA. However, sortilin might also be functionally impaired in the GA, thereby being unable to traffic PrP^{Sc} in the GA to LE/Lys for degradation. The decreased degradation of PrP^{Sc} in LE/Lys and the increased conversion of PrP^{C} into PrP^{Sc} in raft domains or RE could both contribute to the constitutive production of PrP^{Sc} in prion-infected neurons. Dashed arrows indicate restricted trafficking.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