Journal Club

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# The ESCRT Pathway's Role in Prion Diseases and Beyond

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Review of Lawrence et al.

Prion diseases are a special category of brain disorders that have captivated and puzzled neuroscientists. These diseases are caused by an abnormal isoform of prion protein. Under normal circumstances, cellular prion protein ($PrP^{c}$) serves essential roles in maintaining brain health and function. But when misfolded into an isoform first identified in scrapie-infected sheep, prion proteins become infectious agents called $PrP^{Sc}$ (Lawrence et al., 2023). Notably, both neurons and glial cells are affected by prions (Smethurst et al., 2022).

$PrP^{Sc}$ not only fails at its normal job, but also converts healthy $PrP^{C}$ into the toxic form, leading to a self-perpetuating cycle of prion propagation. Furthermore, whereas $PrP^{C}$ is protease-sensitive, $PrP^{Sc}$ contains a protease-resistant core of amino acid residues (Baiardi et al., 2023). Thus, prion infection leads to spreading pathology, including accumulation of proteins tagged with ubiquitin moieties (which target proteins for degradation), synaptic loss, neuronal degeneration, and overgrowth of the postsynaptic density — a characteristic of hyperactive excitatory synapses under pathologic conditions in prion disease (Lawrence et al., 2023). There is no treatment for these diseases, and they are always fatal (Jankovska et al., 2021).

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The aggregation of ubiquitinated proteins (nonfunctional proteins tagged for degradation or recycling) occurs early in prion disease and is believed to play a central role in disease progression. Ubiquitinated proteins are trafficked through the endolysosomal system, and previous studies suggest that disruption of this system plays a central role in prion disease progression. Observable defects in this system within neurons correlate with areas where prions accumulate in the brain (Liberski et al., 2010). Moreover, prion conversion sites, locations where normal prion proteins transform into misfolded, disease-causing forms, have been identified at the plasma membrane and along the endocytic pathway, including early recycling endosomes and multivesicular bodies (MVBs). Therefore, Lawrence et al. (2023) hypothesized that disruption of endosomal sorting complexes required for transport (ESCRTs) is involved in the progression of prion diseases.

The ESCRT pathway plays a major role in the formation of endosomal compartments. It consists of several complexes, including ESCRT-0, -I, -II, -III, and the ATPase VPS4, each playing a distinct role in the formation of intraluminal vesicles (ILVs) and MVBs (Henne et al., 2011). ESCRT-0, comprising hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) and signal transducing adaptor molecule 1 (STAM1), is the first complex in this pathway. It initiates the process by capturing ubiquitinated proteins marked for recycling, disposal, or excretion (Henne et al., 2011) and sequentially recruits ESCRT-I and -II complexes. This leads to cargo clustering and initiation of endosomal membrane invagination, thus forming MVBs.

Some proteins within MVBs enter ILVs, and these are dispatched out of the cell on MVB fusion with the plasma membrane. Once these ILVs exit the cell, they are known as exosomes. Importantly, while the ejection of prions via exosomes successfully removes harmful proteins from the cell, it can jeopardize the health of adjacent cells, which may endocytose the toxic cargoes (Hill, 2019).

Other ubiquitinated proteins in MVBs are routed to the cell's two recycling centers. The primary target is the autophagy-lysosomal system. MVBs fuse with lysosomes to form autolysosomes, with the help of ESCRT-III and VPS4. This fusion creates a unique environment where low pH and abundant hydrolytic enzymes ensure efficient degradation of the proteins (Henne et al., 2011). Alternatively, the cargo of MVBs can be directed to the ubiquitin-proteasome system for degradation (Lawrence et al., 2023).

Depletion of Hrs, one of the two proteins making up ESCRT-0, in neuroblastoma cells infected with prions reduces $PrP^{c}$ to $PrP^{Sc}$ conversion and packaging into MVBs (Lawrence et al., 2023). This suggests that the neuronal ESCRT-0 complex plays a pivotal role in guiding ubiquitinated prions into MVBs for release, degradation, or repackaging. Yet, the full impact of intracellular $PrP^{c}$ to $PrP^{Sc}$ conversion *in vivo*, especially its influence on endosomal maturation in neurons and glial cells, remains unclear. Equally obscure is its connection to the

loss of synapses and the buildup of ubiquitinated proteins.

Aiming to solve these mysteries, Lawrence et al. (2023) used immunoblotting and qRT-PCR to probe Hrs levels in mice inoculated with six different prion strains. They discovered that neuronal Hrs protein levels were lower than normal in infected mice. But neuronal Hrs mRNA transcript levels remained unchanged, suggestive of post-transcriptional dysregulation. Importantly, neuronal Hrs protein levels were also reduced in postmortem brain samples from patients with sporadic Creutzfeldt-Jakob disease, lending clinical relevance to the findings.

To further probe the role of Hrs in prion disease, the authors knocked out Hrs selectively in neurons or glia using the Cre-loxP system. Whereas Hrs depletion in astrocytes or microglia did not significantly affect prion disease progression, its absence in neurons accelerated prion-induced synaptic degeneration and decreased survival time. These effects occurred despite unaltered $PrP^{c}$ to $PrP^{Sc}$ conversion kinetics, suggesting a decoupling of misfolded prion levels and neurotoxicity and challenging the traditional view equating protein misfolding with toxicity.

The authors also probed the role of ESCRT-0 in the sorting of ubiquitinated synaptic proteins and membrane cargo via immunoblotting. In brains of prion-infected mice, loss of neuronal Hrs coincided with a surge in ubiquitinated synaptic proteins, suggesting that neuronal Hrs normally serves a protective role in managing ubiquitinated protein accumulation triggered by $PrP^{Sc}$. However, autophagic-flux, as gauged by p62, LC3-II/I, and LAMP1 immunostaining, remained unaltered in prion-infected Hrs-deficient mice.

In addition, the targeted depletion of neuronal Hrs accelerated several biochemical and structural synaptic aberrations, such as the accumulation of phosphorylated GluA1 receptors, diminished levels of synapsin-1 and mGluR5 receptors, and severe enlargement of postsynaptic compartments assessed through transmission electron microscopy of synaptosomes. These findings suggest that neuronal Hrs depletion may lead to improper handling of ubiquitinated substrates at the synapse. This could potentially trigger a compensatory mechanism, reflected in the changes to receptor levels and synapse structure.

Last, the researchers examined the effects of neuronal Hrs depletion on AMPAR recycling, an essential process for synaptic plasticity often disrupted in neurologic disorders (Zhang and Bramham, 2020). They discovered markedly elevated levels of phosphorylated GluA1 in the end stage of prion disease in prion-infected Hrs-deficient mice. Notably, other studies have linked increased AMPAR phosphorylation to heightened AMPAR activity and synaptic upscaling (Lawrence et al., 2023). Interestingly, the authors found that reducing neuronal Hrs accelerated synaptic degeneration and markedly hastened the progression to the final stages of prion disease. This was coupled with an increase in surface $PrP^{C}$, which may participate in neurotoxic signaling (Lawrence et al., 2023).

Based on these findings, Lawrence et al. (2023) proposed a model in which neuronal Hrs maintains synaptic health and inhibits prion disease progression. When neuronal Hrs, and hence ESCRT-0, is deficient, this exacerbates AMPAR retention, driving heightened excitatory synaptic activity, increased calcium influx, and harmful downstream signaling. This provides novel insights into the ESCRT pathway's neuroprotective role, especially via Hrs, in preventing detrimental synaptic changes, excitotoxicity, and consequent neuronal death.

Another key insight from the work is the suggestion that ESCRT-0 dysfunction through decreased Hrs levels can intensify prion-induced synaptic degeneration independently of an increase in infectious prion proteins. This novel perspective uncovers an intriguing mechanistic decoupling of misfolded prion protein levels and neurotoxicity and suggests that ESCRT-0 plays a vital role in neuroprotection against degeneration.

Importantly, as synaptic dysfunction and protein misfolding are common in neurodegenerative diseases (Soto and Satani, 2011), this information can shed light on similar prion-like neurodegenerative conditions. For instance, an intricate mosaic of biological interactions defines neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD). Among the myriad factors, a prion-like propagation of misfolded proteins, especially in extracellular vesicles such as exosomes, has been gaining attention as a key player (Hill, 2019).

Other potential changes in the ESCRT pathway linked to Hrs alterations remain to be examined. Specifically, exploring prion secretion and uptake via extracellular vesicles, tracked using lipid-specific dyes, such as PKH26, could provide insights into the mechanisms of prion transmission. A deeper exploration of the endosome-to-Golgi retrieval pathway is also warranted because this pathway is critical for prion protein post-translational modification and is known to be disrupted in neurodegenerative conditions, such as AD and PD (Cherry and Gilch, 2020).

Importantly, these findings come at a pivotal point, especially given that numerous clinical trials have targeted protein aggregation in prion diseases, AD and PD, yet they have often been met with disappointment (Baiardi et al., 2023). Mitigating Hrs depletion, and thereby potentially modulating the function of the ESCRT-0 pathway, could slow the progression of these diseases. If proven correct, this pathway could provide a new target for therapeutic interventions. For instance, strategies could be developed to bolster ESCRT-0-mediated targeting of these proteins to MVBs and directing them toward endolysosomal degradation. But this approach must not inadvertently boost exosomal release, as this could paradoxically amplify disease propagation (Hill, 2019).

In conclusion, Lawrence et al. (2023) shed light on how ESCRT-0 dysfunction and synaptic alterations interact in prion diseases. Their revelation of a mechanistic uncoupling between misfolded prion levels and neurotoxicity offers a unique perspective on the intricate interactions within neurodegenerative diseases, departing from traditional views equating protein misfolding directly with neurotoxicity. Their work delineates a new avenue of how cellular pathways, protein misfolding, and synaptic dysfunctions converge in the narrative of CNS degeneration.

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