Final report for CJD Foundation

Investigation of prion inactivation by reactive oxygen species in vivo

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Objectives:

Prion diseases often have extended time periods to clinical disease, indicating that mammals have mechanisms to deal with the pathogenic insult of prion infection, at least for a time. Hypochlorous acid is a reactive oxygen species (ROS) that has been known for decades as a potent antimicrobial. Studies have indicated synthetic HOCl solutions are effective disinfectants for a range of pathogens, including bacteria and viruses, but also prions, tau, and αSyn protein seeds (1). Treatment of prions, tau and αSyn synthetic seeds with HOCl solutions results in conformational changes of the protein seeds that significantly reduce their self-propagating ability (1). In the case of prions, this also eliminates infectivity as assessed by rodent bioassay (1). These prior studies demonstrated evidence that HOCl can act as a potent disinfectant for prions *in vitro*. Our work as part of the CJD research grant builds on these findings to examine the impact of ROS production on prion infection as part of a natural host response *in vivo*. Specifically, our objectives were to investigate how ROS production is employed in lymphoreticular and central nervous system tissues to influence prion sequestration, inactivation and removal following prion infection.

Key findings:

Our key findings indicate that ROS production in a host can alter prion conformations and time to prion disease following infection. However, our studies have revealed that in human neutrophil cultures, ROS production readily alters prions to modify their ability to self-propagate whereas in mouse models the impact of ROS production is more complicated and may be time specific and dependent on the route of infection and tissue infected. These findings warrant further investigation, and we continue to pursue these studies. Our main findings, currently being prepared for peer-reviewed publication, are detailed below.

1) ROS production by human neutrophils alters the self-propagating capabilities (i.e. seeding activity) of prions. Neutrophils with higher levels of ROS production are more efficient in reducing prion seeding activities.
2) Specific biomarkers of HOCl modifications occurred with prion deposits in the brain tissue of prion-diseased animals, suggesting that prions have been modified by the

same HOCl modifications that are known to reduce prion self-propagating capacities *in vitro*.

3) Our evidence indicates that genetic mouse models with altered ROS production capabilities, including those deficient in producing HOCl, have altered times to clinical disease after prion infection compared to wild-type animals. However, our data indicates that the impact of ROS on prions may differ depending on the infected tissue, route of exposure, and time since prion infection. Timely ROS production is a critical component of cellular defense systems to deal with pathogens, yet unchecked ROS production has a myriad of detrimental effects such as oxidative damage and cell death. We continue to study the impacts of ROS production at pre-clinical and clinical stages of disease following different routes of prion infection.
4) We have detailed a time course examining the distribution and quantity of prions that occur 48 hours to ~180 days post-infection in peripheral tissues and the central nervous system. These studies were completed in mouse models with altered ROS production and compared to normal (wild-type) animals. We continue to study how host responses, including differences in ROS production, impact the distribution and quantity of prions.

Understanding how ROS modifications impact the amplification capacity of prions in diseased states is critical to understand the impact of ROS in prion and other neurodegenerative diseases. These mechanistic insights will help evaluate the potential benefits of targeting ROS mechanisms as therapeutic avenues.

Next steps:

I began to study the impact of ROS on prion infection in the laboratory of Dr. Byron Caughey at Rocky Mountain Laboratories. In 2019, I transitioned to my own independent position as Assistant Professor at Case Western Reserve University (CWRU) where I continue to study prion diseases and other neurodegenerative diseases. This includes work to further examine ROS impact in the context of human prion diseases in collaboration with the National Prion Disease Pathology Surveillance Center at CWRU.

The potent anti-amyloid activity of HOCl *in vitro* may suggest HOCl production *in vivo* could provide a more general innate defense mechanism to remove misfolded proteins. Specific ROS biomarkers are known to occur in Alzheimer's disease (AD) and HOCl-related modifications are reported to occur on amyloid β (Aβ) aggregates in AD (2) and αSyn in PD (3). However, if and how these modifications influence the self-propagating capacity of Aβ, tau, and αSyn aggregates *in vivo* is unknown. We continue to investigate how specific ROS modifications occur on disease-related tau and αSyn aggregates in AD and PD and correlate this to misfolded protein accumulation and pathogenesis.

Other relevant recent work:

High resolution structures of prions have remained elusive for decades, a knowledge gap that has severely limited our molecular understanding of prions and how they propagate to result in disease. Using state-of-the-art cryogenic electron microscopy techniques, we recently determined the first high resolution structure of an infectious mammalian prion in a collaboration between CWRU and RML (Figure 1, (4)). This structure provides the first atomic resolution level of insight to detail a molecular basis for how prions propagate to cause disease and to further support rational structure-based drug design. To expedite reporting of these findings to the scientific community while the study undergoes peer-review, we recently posted our data on BioRxiv (4). My lab continues to study the molecular underpinnings of prion structures including,

although not limited to, how ROS-modifications may alter the highly organized architectures of prions.

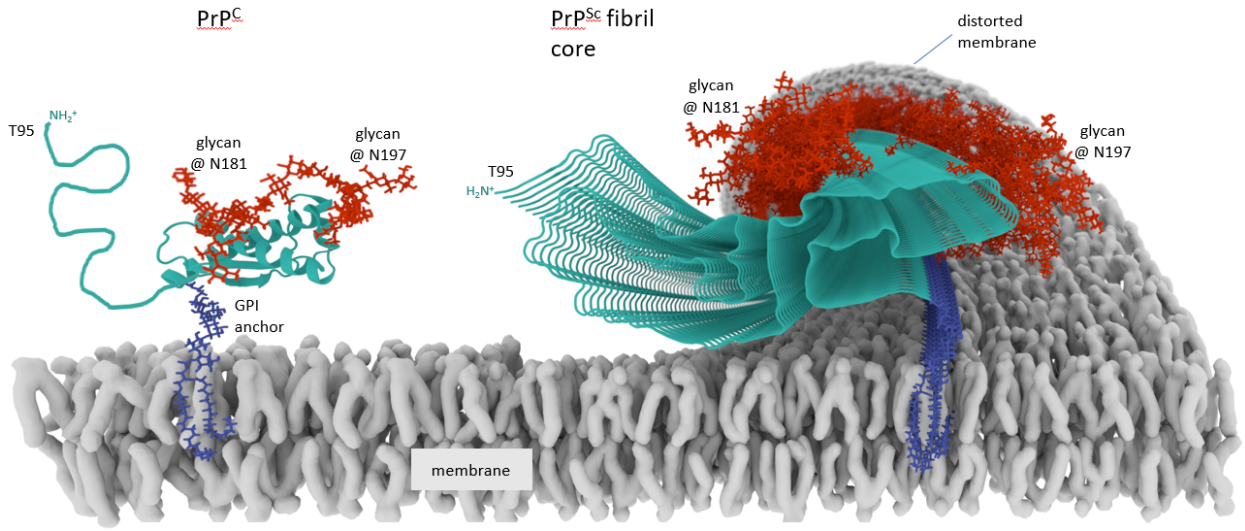

Figure 1. High resolution structures of infectious mammalian prion (PrP^{Sc}) fibrils are depicted beside normal membrane-bound PrP^{C} (residues 95-231). The ordered fibril core (turquoise) determined by cryogenic electron microscopy is shown with hypothetical illustrations of glycans (red) and GPI anchors (blue) that were unresolved in the cryo-EM images. These findings were recently reported at (4).

References

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