Final Report: 2023-2024 Postdoctoral Fellowship

Project Title:

High-resolution structure determination of MM1 and MM2 sCJD prions

Project Objective:

The main objective of this project is to determine the molecular structure of two strains of human sCJD prions, MM1 and MM2. These two types of prion protein filaments, although share the same amino acid sequence, have been shown to have different pathological properties. Numerous previous studies including ours suggested that this difference is associated with the different 3D structures between MM1 and MM2 prion filaments. At the time of discovery, it was very difficult to isolate highly purified human prions, and the then conventional methods of structural biology were not suitable for determining the 3D structure of protein aggregates like prion filaments. However, recent progress in cryogenic-electron microscopy (cryo-EM) allowed high-resolution structure determination of most proteins, including prions. Thus, we aim to use this state-of-the-art method to determine the high-resolution structure of prion filaments extracted from MM1 and MM2 type sCJD patients.

Project Achievements:

We have acquired the brain tissue of 3 patients with MM1 type sCJD, and 3 patients with MM2 type sCJD. Western blotting has confirmed that they all contain PK-resistant prion protein (PrP^{Sc}), and the PK-resistant core correlates with the signature molecular weight of MM1 and MM2 types of sCJD prions. We started the purification of these human prions using a previously established protocol (Wenborn et al., *Scientific Report*, 2015) optimized for extracting prions from lab animals. This protocol utilizes tungsten salt to precipitate prion filaments, and has been proven to yield high-quality samples that are suitable for structural studies. From this standard protocol, we were able to extract prion rods, but this extract is also accompanied by other amorphous aggregates, and spherical particles. Unfortunately, samples like this will not be suitable for further high-resolution structural studies. Thus, we mainly focused on modifying the protocol for prion rod purification. We have achieved better purity by adding more rounds of centrifugation, but the sample quality still needs to be further improved. Nevertheless, more studies are needed to study the identity, pathological features, and structural features of these non-fibrilar components.

At the same time, we found this protocol can be used to purify high-quality cervid prions. These prions affect deers in nature, but their transmission to humans has not been well studied. We previously established a method to predict cross-species transmission using their structures (Li et al., *Nature Structural and Molecular Biology*, 2022), and hope to understand the cross-species transmission of these deer prions. Currently, we have determined a 7-8 Å structure. From this structure, we noticed that deer prions adopt quite different structures than those from lab animals (rodents), emphasizing the importance of using real patient-derived samples for structural studies of human prions. This would again highlight the importance of establishing an effective protocol for purifying high-quality human prions as mentioned earlier.

Key Findings and Implications for the Prion Disease Field:

From our literature search during the project, we were surprised that there is no established protocol to isolate highly purified prion filaments from the brains of CJD patients. Our studies suggested conventional tungsten precipitation will yield large amounts of impurities. Thus, one should take extra precautions when interpreting studies involving purified prions. Our effort

would also be a new standard for direct studies of purified human prion, avoiding complications of animal models.

Our studies involving deer prions also suggested that prions from different species adopt different structures, and there is far more to be resolved in the field of structural biology. Again, the attempt to establish an effective protocol will be a key starting point.

Next Steps:

We do have plans to finish the two projects mentioned above: 1) to establish the purification protocol for human prions, to extract highly purified samples from human brains; this will lead to the subsequent structural studies of MM1 and MM2 prions, which will complete this planned project; 2) we will finish the final refinement of deer prions and published the detailed molecular structure, potentially reveal the impact of this disease on humans.