138. Human cerebral organoids propagate sporadic CJD prions

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ABSTRACT

Introduction: Human prion disease has traditionally been difficult to model in cell culture. Very few human cell lines show sustained prion propagation over time and none represent three-dimensional (3D) brain tissue. Recently it was found that astrocyte cultures differentiated from human induced pluripotent stem cells (Hu-iPSCs) take up and propagate sporadic CJD (sCJD) prions with the resulting infection influenced by the sCJD subtype [1]. Hu-iPSCs have also been utilized to differentiate self-patterning, structured human brain tissues called cerebral organoids [2,3]. We hypothesized that these cerebral organoids could be used to model human prion propagation *in vitro*.

Materials and Methods: Cerebral organoid cultures were generated from hu-iPSCs using the protocol developed by Lancaster and Knoblich [2]. Cerebral organoids were infected with one of two sCJD inocula and monitored for health, morphology, PrP seeding activity, PrP deposition and proteinase-k resistant PrP as compared with organoids that received a normal brain homogenate inoculum.

Results: Cerebral organoids showed uptake and propagation of prions, detected by RT-QuIC analysis, following exposure to either sCJD inocula. Inoculum specific differences were observed. One inoculum showed robust proteinase-K resistant PrP in all tested organoids and coarse, granular PrP staining in the organoid interiors. The other inoculum induced a more toxic phenotype that affected organoid health but displayed a low level of prion seeding activity with no detectible protease-resistant PrP.

Conclusions: Cerebral organoid cultures can be used to model human prion disease *in vitro*. These structured 3D mini-tissues offer a capacity to investigate aspects of prion diseases never previously available in a human neuronal cell model. Potential human disease-specific applications include delineating sCJD subtype pathogenesis and testing putative therapeutics.

KEYWORDS: Cerebral organoid; sporadic CJD; induced pluripotent stem cell; RT-QuIC

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139. Cryo-electron microscopy of chronic wasting disease prions

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ABSTRACT

Introduction: Chronic wasting disease (CWD) is a fatal neurodegenerative disease in cervids that belongs to a group of diseases known as prion diseases. CWD has been detected in Canada, United States, South Korea, Norway, and most recently in Finland. CWD is the most contagious prion disease and poses a risk for transmission to other species including humans.

Aim: PrP^{Sc} is the misfolded form of the cellular prion protein (PrP^{C}) and the infectious agent that causes prion diseases. The molecular mechanism of PrP^{C} to PrP^{Sc} transformation is unknown. To understand this structural conversion, it is essential to understand the structure of PrP^{Sc}. Thus, the goal of this project is to investigate the structure of PrP^{Sc} forms that are causing CWD, using negative stain and cryo electron microscopy (EM).

Method: PrP^{Sc} amyloid fibrils were purified from the brains of CWD-infected Tg33 mice, which express deer prion

protein. To purify PrP^{Sc} amyloid fibrils, phosphotungstate anions and sarkosyl were used. In addition, enzymes such as proteinase K (PK) or pronase E (PE) were added to remove PrP^{C}. To assess the quality and quantity of the PrP^{Sc} fibrils, the purified amyloid fibrils were negatively stained and visualized using EM. Cryo-EM and 3D fibril reconstruction are then performed on fibril preparations.
Results and Conclusion: PrP^{Sc} amyloid fibrils were successfully purified and visualized using negative stain EM. Both, PK- and PE-purified samples displayed complex yet similar morphologies. However, a dominant and recognizable type seen in both preparations consisted of filaments with a clear twisted ribbon-like morphology. 2D class averages and 3D reconstructions from the negatively stained micrographs could help to better classify these fibrils. Additionally, cryo-EM analyses could resolve the structural differences between conformers of distinct CWD prion strains and their fibrillization properties. PE-purified fibrils, on the other hand, exhibited striations that run perpendicular to the fibril axis. Interestingly, the distance between these striations is ~40 nm, which corresponds to the molecular height of two PrP^{Sc} molecules in a four-rung beta solenoid architecture that may adopt a head-to-head arrangement [1]. This observation could be explained by the presence of sarkosyl molecules associating with the hydrophobic GPI-anchor of the stacked PrP^{Sc} molecules. Further image processing analyses will provide a more complete understanding of the orientation of the GPI-anchor in the CWD fibrils.

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140. Molecular dynamics simulations of cervid prion protein variants to assess protein stability and susceptibility towards chronic wasting disease

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ABSTRACT

Introduction: Chronic wasting disease (CWD) is a prion disease that affects cervids. The central event in prion diseases is the structural conversion of the healthy, cellular prion protein (PrPC) to the disease-causing isoform, PrPSc. It is technically difficult to experimentally study the initial steps of the PrPC to PrPSc conversion; however, molecular dynamics (MD) simulation plays an important role in probing the misfolding events. One approach is to assess the effect of single-residue substitution on the structural stability of PrPC. Polymorphisms in the prion protein gene can influence prion disease susceptibility, disease progression, clinical presentation, and the propagation of distinct CWD strains associated with different PrPSc conformations. In this project, we use molecular dynamics simulation as a tool to characterize the effect of white-tailed deer (WTD) prion protein polymorphisms Q95H and G96S on the experimentally determined structure of the natively folded prion protein.
Methods: The initial model for the WTD prion protein (residues 93–233) was generated using the crystal structure of recombinant deer prion protein (PDB: 4YXH) as the template. The wild type (WT), 95H, and 96S models were subjected to minimizations, equilibrations, and production MD simulations using the Gromacs package. In total, three independent 50ns simulations were performed for each system.
Results: The 96S polymorphism displayed a higher root mean square deviation (RMSD) and root mean square fluctuation (RMSF) values, which are indicators of lower structural stability compared to WT and 95H forms. Also, 96S had a larger radius of gyration (Rg) values, indicating that the structure of 96S was less compactly folded than WT and 95H. Calculating the solvent accessible surface area (SASA) of 96S revealed that the structure of 96S was more exposed to the solvent. On the other hand, structural dynamics of the 95H polymorphism were closer to WT. RMSF and Rg values for 95H indicated higher stability and compactness of its structure. Additionally, the SASA values of 95H were very similar to WT.
Conclusions: Experimentally, the 96S allele is associated with slower disease progression and animals carrying this polymorphism have longer incubation time when inoculated with CWD prions. According to our MD results, it is apparent that the structure of WTD prion protein carrying the 96S polymorphism is less stable than the WT protein. The clear difference in the structural stability and local dynamics of 96S