CJD Foundation award mid-project update

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Project title: Assessing efficacy of zinc-finger repressors of prion protein as a therapeutic for prion disease

Project objective: All cases of prion disease are caused a single protein, the prion protein or PrP. Lowering the amount of PrP in the brain has been shown to be protective against prion disease. Our lab is interested in exploring different kinds of drugs that could have this effect. The goal of this project is to test zinc finger proteins, or ZFPs, that are targeted at the prion protein gene. ZFPs can be thought of as a form of gene therapy, in which instructions for making a ZFP are delivered into a cell using a special package called a viral vector. Inside the cell, ZFPs are produced that specifically recognize the prion protein gene, and physically sit on that gene in order to prevent production of its RNA and protein. In collaboration with our partners at Sangamo Therapeutics, we aim to test a range of ZFPs against mouse and human PrP, first in cells, then in mice, to test how potently they reduce PrP. The final stage of the project will be testing the top PrP-lowering ZFPs in prion-infected mice to understand their impact on the disease process.

Accomplishments to date:

1. **Screening**: More than 200 ZFPs targeting the prion protein gene were screened in cells. This was done for both the mouse and human prion protein genes.
2. **Confirmation of lead compounds:** The several best ZFPs per species were further tested in cells, confirming potency and dose-responsiveness. These best compounds were able to reduce PrP by more than 90%
3. **Potency in mice**: Next, the best compounds were tested in mice bearing the appropriate prion protein gene (either mouse or human.) This required packaging the ZFPs in viral vectors, delivering by an injection into the tail, then analyzing the brain one month later. The top candidates were able to reduce PrP across the brain by 50%. Of note, the viral vector/ that we used targets neurons in particular, which are the most important cell type in prion disease.
4. **Efficacy in mice**: We have now launched a survival study in which prion-infected mice will receive ZFP treatment at either an early or late stage of disease. In addition to measuring symptoms and survival, we will periodically measure a marker of neuronal damage in blood to understand how it responds to treatment.

Key findings and implications for the prion field: While this project is ongoing, we have already learned that ZFPs are able to potently lower PrP levels. In the cells that they reach, ZFPs appear able to basically turn off PrP production, as shown by very strong (>90%) repression when treating cells in a dish. The key issue for effective treatment in an animal or person, therefore, will be reaching as many cells as possible in the living brain. Our mouse studies so far have used a mouse-specific viral vector, and with this tool we appear to be lowering PrP in the mouse brain by 50%, a promising level of reduction that we believe is biased towards neurons, the cell type we care about most in prion disease.

Next steps: We are testing now the level of survival benefit conveyed by this reduction in prion-infected mice. In the bigger picture, as viral vector technology advances, the major open question for this project and its clinical prospects is whether a viral vector exists, or can be identified, that is able to achieve comparable delivery in the human brain. The good news is that many smart people are working on this problem, which is of interest not just for prion disease but for a wide range of brain diseases.