CJD Foundation Grant Final Report

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Title: The RAP approach for effective prevention and treatment of prion diseases

Project objective

We aim to develop an effective gene therapy treatment and prevention strategy against human prion diseases through simultaneously knocking down the key mediator for prion toxicity, suppressing the replication of the prion agents, and protecting brain cells from prion-induced toxicity and damages.

Summary of accomplishments to date:

We have successfully developed several plasmid DNA vectors that can knock down the expression of the gene for human prion protein (PrP), the essential mediator for prion toxicity and substrate for prion replication. We have also developed a plasmid vector that expresses a secreted form of a neuroprotective PrP peptide (N1+), which can protect neuronal cells from prion toxicity and other cellular stresses. We have made rAAV viral particles from the above plasmids that will either knock down the PrP gene (rAAV-knockdown) or overexpress the neuroprotective N1+ PrP peptide (rAAV-N1+). We found evidence that the neuroprotective PrP N1 peptide has direct anti-prion replication activity in vitro. The first experiments in a transgenic mouse model overexpressing human PrP (Tg40h) using these plasmid vectors assisted by novel nanoparticles or rAAV viral particles are completed.

Key findings and implications for the prion disease field

The plasmid vectors and the corresponding rAAV viral particles we developed for PrP gene knockdown or expression of the neuroprotective PrP N1+ peptide all appear to be safe for gene therapy treatment in mice inoculated with a human CJD prion, which fulfills a key safety requirement for future clinical trials.

In the Tg40h humanized mouse mice intracerebrally inoculated with the most common CJD strain, a single intraventricular infusion with a plasmid expressing the N1+ peptide (in nanoparticle forms) three weeks before intracerebral CJD infection failed to extend the survival time when compared with mice similarly treated with a control plasmid expressing GFP (226±6 days vs. 233± 6 days). Oral doxycycline treatment starting at 9 days after CJD inoculation for mice infused with the N1+ plasmid earlier led to a slight decrease in survival time ((216±10 days vs. 226± 6 days), which is contrary to expectation. These results suggest that doxycycline is not effective against human CJD prions and that the current N1+ plasmid nanoparticles may not have achieved sustained N1+ expression in the brain.

In the same Tg40h humanized mouse model that were intracerebrally inoculated with the most common CJD strain, after a single retro-orbital infusion with rAAV-knockdown and/or rAAV-N1+ (packaged with the B10 capsid) at 157 days post CJD inoculation (around clinical onset), the rAAV-knockdown treated mice had a modest 9.6% increase in survival time (216±3 days vs. 197±3 days)(p-0.000304), but neither the rAAV-N1+ treated mice nor the rAAV-knockdown/rAAV-N1+ treated mice showed significant extension of survival. The Tg40h mice overexpress human PrP, which makes it much harder to knock down the PrP expression to a level that can influence the survival time. Therefore, the modest extension of survival by the rAAV-knockdown treatment at clinical onset for CJD in the human PrP-overexpressing Tg40h mice is highly significant. We believe that when tested in a mouse model expressing human PrP at the normal level or in human patients, treatment with the rAAV-knockdown at early clinical phase will be effective in extending survival as well as improving life quality for CJD patients. We think that preclinical treatment with rAAV-knockdown will prevent or at least significantly delay the onset of clinical disease in spontaneous mouse models of prion diseases and in human patients carrying pathogenic PrP mutations.

Next steps and related prion research

We will examine the rAAV-knockdown and/or rAAV-N1+ treated mice for signs of attenuated pathology and/or reduced prion deposition in the brain. We will study whether N1+ expression from a different vector will achieve synergistic effect when used together with rAAV-knockdown. We will conduct similar experiments in mouse models that express human PrP at the normal level and in transgenic mouse models that develop a spontaneous human prion disease, which will better mimic prion diseases in humans. We will treat these mice at various preclinical and clinical stages with our plasmid or viral vectors to assess the effectiveness in prevention and treatment of human prion diseases. In addition, we will further optimize the plasmid vectors to develop a naked DNA vector capable of sustained cargo gene expression to replace viral vectors that are extremely expensive and carry other major caveats.