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Research Article

Conditional Deletion of *Prnp* Rescues Behavioral and Synaptic Deficits after Disease Onset in Transgenic Alzheimer's Disease

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ABSTRACT

Biochemical and genetic evidence implicate soluble oligomeric amyloid-beta (Aβo) in triggering Alzheimer disease (AD) pathophysiology. Moreover, constitutive deletion of the Aβo-binding cellular prion protein (PrP^C) prevents development of memory deficits in APP_{swe}/PS1ΔE9, a model of familial AD. Here, we define the role of PrP^C to rescue or halt established AD endophenotypes in a therapeutic disease-modifying time window after symptom onset. Deletion of *Prnp* at either 12 or 16 months of age fully reverses hippocampal synapse loss, and completely rescues pre-existing behavioral deficits by 17 months. In contrast, but consistent with a neuronal function for Aβo/PrP^C signaling, plaque density, microgliosis and astrocytosis are not altered. Degeneration of catecholaminergic neurons was unchanged by PrP^C reduction after disease onset. These results define the potential of targeting PrP^C as a disease-modifying therapy for certain AD-related phenotypes after disease onset.

Keywords: Alzheimer disease; cellular prion protein; tamoxifen inducible Cre-lox system

Abbreviations: APP_{Swe}/PS1ΔE9 = Amyloid precursor protein with familial Swedish mutation/Presenilin 1 exon 9 deletion; Aβ = amyloid-beta; Aβo = amyloid-beta oligomers; PrP^C = cellular prion protein; *Prnp* = prion gene name

SIGNIFICANCE STATEMENT

The study presented here further elucidates our understanding of the Aβo-PrP^C signaling pathway in a familial form of Alzheimer disease (AD) by implicating PrP^C as a potential therapeutic target for AD. In particular, genetic deletion of *Prnp* rescued several familial AD (FAD) associated

phenotypes after disease onset in a mouse model of FAD. This study underscores the therapeutic potential of PrP^C deletion given that patients already present symptoms at the time of diagnosis.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia worldwide with more than 5 million Americans with the disease (Alzheimer's, 2012). AD is characterized by two hallmark pathologies: amyloid-β (Aβ) plaques composed of Aβ peptide and neurofibrillary tangles composed of hyperphosphorylated Tau (Braak and Braak, 1991; Selkoe, 2011). The clinical presentation of AD is characterized by progressive memory loss and early death (Molsa et al., 1986; Mayeux, 2003). Central to AD is the inability of patients to form new memories, with synaptic dysfunction and loss being tightly correlated with symptom progression (Selkoe, 2002; Scheff et al., 2006). Thus understanding how synapses are lost is key to understanding AD. Genetic and biochemical evidence suggest a soluble high-molecular weight oligomeric amyloid-β peptide (Aβ as) a trigger for synaptic dysfunction in AD (Hardy and Selkoe, 2002; Sheng et al., 2012; Dohler et al., 2014; Kostylev et al., 2015). Several studies in rodent models have shown that Aβ can initiate a cascade of deleterious effects on synaptic function (Lambert et al., 1998; Walsh et al., 2002; Lesne et al., 2006; Shankar et al., 2008). These studies highlight the importance of understanding Aβ-dependent synaptotoxicity.

Recent evidence suggests PrP^C as a central protein in mediating synaptotoxicity. Previous work has shown PrP^C as a high-affinity binding partner of Aβ and mediator in suppressing LTP (Lauren et al., 2009). Additionally, constitutive deletion of *Prnp* can rescue synapse density, survival, and learning and memory deficits seen in a mouse model of familial AD (Gimbel et al., 2010). Other

groups have shown PrP^C to bind Aβ with high affinity (Chen et al., 2010; Dohler et al., 2014), to mediate suppression of LTP (Barry et al., 2011; Freir et al., 2011; Klyubin et al., 2014), and suppress learning and memory (Chung et al., 2010; Fluharty et al., 2013). Nevertheless, conflicting reports of the role for PrP^C in mediating synaptotoxicity (Balducci et al., 2010; Calella et al., 2010; Kessels et al., 2010) have prompted further studies to test the therapeutic potential of PrP^C as a target.

Targeting PrP^C for AD treatment holds the potential for disease-modifying therapy, as opposed to the symptomatic action of current interventions approved for AD (Yiannopoulou and Papageorgiou, 2013). Several studies have shown directly or indirectly that Aβ binding to PrP^C leads to PrP^C-mGluR5 coupling (Um et al., 2013; Haas et al., 2015) and subsequent activation of intracellular components including eEF2 (Um et al., 2013; Ma et al., 2014) and Fyn (Larson et al., 2012; Um et al., 2012; Rushworth et al., 2013; Kaufman et al., 2015) can lead to dendritic spine loss (Um et al., 2012; Zhang et al., 2015), suppressed synaptic plasticity (Hu et al., 2014; Haas et al., 2015), and Tau phosphorylation (Larson et al., 2012; Kaufman et al., 2015). Multiple groups have begun to develop methods to target the Aβ-PrP^C interaction using small molecule approaches (Fluharty et al., 2013; Aimi et al., 2015; Osborne et al., 2016) and immunotherapy approaches (Chung et al., 2010; Barry et al., 2011). These efforts underscore the need to understand whether the Aβ-PrP^C interaction is required for AD phenotype maintenance and progression after disease onset.

In order to test the therapeutic potential of targeting *Prnp*, we decided to take advantage of a tamoxifen (TMX) inducible Cre-lox system to partially delete *Prnp* early after disease onset, and months after disease onset in a mouse model of familial AD. Partial deletion of *Prnp* was able to

rescue synaptic and behavioral deficits in a mouse model of AD at 12 and 16 months. These results highlight the clinical potential of targeting the Aβ-PrP^{C} interaction.

MATERIALS AND METHODS

Animals

All mice were cared for by the Yale Animal Resource Center. Yale's institutional animal care and use committee approved all experiments. As previously described (Gimbel et al., 2010) the mouse strains used were the APP_{swe} / PSEN1$_{dE9}$ mice on a C57BL/6J background, and the ER-Cre mice (Hayashi and McMahon, 2002) on a C57BL/6J background were purchased from Jackson Laboratory (Bar Harbor, ME). The flox-*Prnp* mice on a C57Bl6 background have been described (Tuzi et al., 2004; Bradford et al., 2009). All experiments utilized littermate control mice. The 12MD cohort contained a 2:1 male to female sex ratio while the 16MD cohort contained a 1.1:1 male to female sex ratio. The differential male to female sex ratios was not intentional but a cause of random breeding and selection.

Brain tissue collection

Immunohistology

Mice were euthanized and immediately perfused with ice-cold phosphate buffer saline (PBS) for two minutes, followed by a five-minute perfusion with ice-cold 4% paraformaldehyde (PFA). Brains were dissected out, cut down the midline into two hemispheres and fixed for 24 hours in 4% PFA. Following fixation, brains were cut into 40 µm parasagittal sections using a Leica (Wetzlar, Germany) WT1000S Vibratome. Sections were stored in PBS at 4ºC until staining.