Amyloid-β induced signaling by cellular prion protein and Fyn kinase in Alzheimer disease

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Keywords: prion, Fyn kinase, Alzheimer disease, PrpC, cellular prion protein

Alzheimer disease (AD) is the most prevalent cause of age-related dementia, affecting more than 35 million people worldwide, and potentially 115 million people by 2050. The histopathological hallmarks of AD are extracellular amyloid β (Aβ) peptide aggregates or “amyloid plaques” and neurofibrillary tangles (NFTs) containing the microtubule-associated protein, tau. The histological features are associated with neurodegeneration and cognitive decline, leading eventually to death.

The leading hypothesis for AD postulates causation by Aβ peptide (especially Aβ42), which is derived proteolytically from amyloid precursor protein (APP) by the action of β- and γ-secretases. Aβ peptide can exist as soluble monomers, soluble oligomers, intermediate protofibrils and insoluble fibrillar aggregates. Among these Aβ species, cognitive impairment and the degree of synaptic deficit in the AD brain are most strongly correlated with soluble oligomeric assemblies of Aβ, suggesting that soluble Aβ oligomers trigger neuronal toxicity to cause dementia.

Identification of PrPC as a High-Affinity Receptor for Aβ Oligomers

Cellular prion protein (PrPC) was identified as a high affinity binding site of Aβ by a genome-wide unbiased expression cloning. After the binding affinity is corrected for the extent of oligomerization, the estimated affinity of oligomeric Aβ is 1–10 nM. In vitro and in vivo studies have confirmed a direct and oligomer-specific high affinity interaction of soluble Aβ oligomer with PrPC.

PrPC is a membrane-anchored neuronal glycoprotein whose normal function is uncertain. For a class of fatal neurodegenerative disorders affecting human and animals, termed prion diseases or transmissible spongiform encephalopathies (TSEs), PrPC is required. In these conditions, normally folded endogenous PrPC undergoes a transformation to a conformationally altered scrapie prion protein (PrPSc) that accumulates in the brain as insoluble aggregates. This process leads to neuronal dysfunction and progressive neurodegeneration, for which there is no clinically effective treatment. PrPC mediates toxic signaling of PrPSc by its binding to β-sheet rich conformers of PrPSc. The oligomeric state of Aβ recognized by PrPC may share conformational properties of other misfolded proteins such as PrPSc-bound to PrPC.

Definition of the molecular and cellular consequences of Aβ-PrPC complex formation provides the opportunity to advance understanding of AD pathophysiology.

Inhibition of LTP by Aβ Oligomer is Mediated by PrPC

Hippocampal long-term potentiation (LTP), a leading experimental model for the synaptic changes underlying learning and memory, is strongly inhibited by Aβ oligomer application, as reported by numerous laboratories. Aβ oligomer-induced LTP blockade is absent in the hippocampal slices from Prnp−/− mice, and those anti-PrPC antibodies preventing Aβ oligomer binding to PrPC rescue synaptic plasticity in hippocampal slices from oligomeric Aβ. Thus, disruption of synaptic plasticity by soluble Aβ oligomers is mediated through PrPC under these conditions.

However, Kessels et al. observed Aβ42 suppression of hippocampal CA1 LTP in slices from Prnp−/− mice. Furthermore, the impairment of hippocampal synaptic plasticity in one AD mouse model was not altered by ablation or overexpression of PrPC. These findings challenged the role of PrPC as a mediator of Aβ oligomer toxicity. In this context, it is important to recognize that Aβ preparations vary greatly in composition, and this variability may explain variable outcomes in functional experiments. For this reason, a careful characterization of Aβ oligomers is required by size exclusion chromatography, electron microscopy and atomic force microscopy, as well as SDS-PAGE. Without such characterization, there is a potential for Aβ to elicit nonspecific toxicity, bypassing the need for any
specific neuronal receptor, including PrP\textsuperscript{C}. It is critical to evaluate the action of well-characterized A\textsubscript{B} species, including those derived from human AD brains, as being most relevant for AD pathophysiology. With synthetic A\textsubscript{B} oligomers resembling those of the original study,\textsuperscript{8} the requirement for PrP\textsuperscript{C} to mediate LTP inhibition has been verified.\textsuperscript{11} In addition, two studies showed that Tris-soluble extracts from human AD brain, which contain disease-relevant A\textsubscript{B} oligomers, require PrP\textsuperscript{C} for inhibition of LTP in mouse brain slices.\textsuperscript{12,18} One of these studies included in vivo electrophysiological outcomes. A\textsubscript{B} oligomers derived from human AD brain were injected into the rat brain and LTP was measured. Unlike the synthetic A\textsubscript{B} oligomer preparation used by studies from Kessel et al., which caused a marked reduction in baseline excitatory synaptic transmission,\textsuperscript{16} the A\textsubscript{B}-containing AD brain extract selectively inhibits LTP in vivo. The in vivo inhibition of LTP by A\textsubscript{B} oligomer is fully alleviated by preinjection into the hippocampus of anti-PrP\textsuperscript{C} antibodies that recognize the A\textsubscript{B} binding domain in PrP\textsuperscript{C}. In addition to supporting the earlier finding that PrP\textsuperscript{C} functions as a receptor for mediating toxicity of A\textsubscript{B} oligomer, these data provide evidence to support a biological, antibody-based approach to PrP\textsuperscript{C} as a target for AD.

**Role of PrP\textsuperscript{C} in Synaptic Loss**

A hallmark of AD is the massive synaptic loss that occurs at an early stage of the disease.\textsuperscript{19} Synapse loss can also be detected in APP/PS1 mice by staining for synaptic marker proteins, and the loss of synaptic markers is fully dependent on PrP\textsuperscript{C}.\textsuperscript{20} Treatment of aged APP/PS1 mice with anti-PrP\textsuperscript{C} antibodies allows a recovery of depleted synaptic density in the dentate gyrus.\textsuperscript{21} In vitro studies have described dendritic spine loss after acute A\textsubscript{B} oligomer exposure. Imaging of spines continuously over 6h shows that A\textsubscript{B} oligomer treatment of dissociated hippocampal neurons induces a 10–15% loss of spines, but Prnp\textsuperscript{-/–} cultures are fully protected.\textsuperscript{22}

It has been known that A\textsubscript{B} oligomer binding sites localize to the postsynaptic density,\textsuperscript{23,24} consistent with their inducing synaptic loss. To the extent that PrP\textsuperscript{C} mediates A\textsubscript{B} oligomer effects on spine composition, morphology and density, the protein is predicted to be concentrated at synapses and present in the postsynaptic density. Biochemical subcellular fractionation, immunohistological studies and unbiased proteomic studies\textsuperscript{22,25} show that PrP\textsuperscript{C} is located at synapses and enriched at postsynaptic densities.

**Role of PrP\textsuperscript{C} in Rodent Memory Impairment**

The experimental paradigm most closely related to human AD is analysis of rodent memory. Mouse genetic models expressing familial AD mutant APP with or without familial AD mutant PS1 are useful tools for the study of AD. Transgenic mice expressing APP mutations exhibit age-dependent deficits of spatial learning and memory in water maze studies.\textsuperscript{26,27} Injection of A\textsubscript{B} oligomer isolated from transgenic mice, human- or cell-derived A\textsubscript{B} oligomers into naïve mice produces learning and memory impairment.\textsuperscript{28,29} Balducci and colleagues injected synthetic A\textsubscript{B} oligomer into the hippocampus and assessed novel object recognition with and without PrP\textsuperscript{C}.\textsuperscript{31} This model is simple, but relies on synthetic peptide and on acute effects. A\textsubscript{B} oligomer injection prevented novel object recognition memory by wild type mice. In A\textsubscript{B}-injected mice lacking PrP\textsuperscript{C}, the recognition of novel vs. familiar objects was preserved, but mice preferred familiar objects rather than novel objects.\textsuperscript{11} This reversal complicates any assessment of the role for PrP\textsuperscript{C} in A\textsubscript{B} action using this paradigm.

The most direct assessment of PrP\textsuperscript{C} in AD-related deficits entails measuring spatial learning and memory in mice carrying familial AD genes but null for PrP\textsuperscript{C} expression. Mice lacking PrP\textsuperscript{C}, but containing A\textsubscript{B} plaque derived from APP\textsuperscript{swe}/PS1\textsuperscript{ΔE9} transgenes, show no detectable impairment of spatial learning and memory.\textsuperscript{20} Furthermore, the treatment of aged APP\textsuperscript{swe}/PS1-M146L mice with anti-PrP\textsuperscript{C} antibodies reverses memory impairments.\textsuperscript{21} These data suggest that memory deficits in AD transgenic mice require the presence of PrP\textsuperscript{C}. However, Cisse et al. reported that PrP\textsuperscript{C} is not essential for this phenotype in the J20 line.\textsuperscript{30} Discrepancies between different animal AD models with respect to a possible role for PrP\textsuperscript{C} might be anticipated. The many reported animal models of AD are each likely to recapitulate only part of the human syndrome. While PrP\textsuperscript{C} appears to be an essential receptor for certain toxic A\textsubscript{B} species, it would not be expected that PrP\textsuperscript{C} ablation to rescue all aspects of pathology in each model. The most critical difference between these studies is the AD transgenic line investigated, and the age of onset for behavioral deficits. Critically, the J20 mice are known to be memory-impaired as young adults, perhaps due to the high juvenile A\textsubscript{B} levels in this strain.\textsuperscript{31,32} There is no evidence for progressive memory loss in these mice after normal development, as occurs in AD. In contrast, the APP/PS1 lines, which show PrP\textsuperscript{C} dependence of memory dysfunction, possess normal spatial memory at 3–6 months of age.\textsuperscript{20,33} The most plausible conclusion is that PrP\textsuperscript{C} is required for adult-onset AD transgene-driven progressive disease, whereas developmental-onset deficits in the J20 occur by a different mechanism. The developmental disorder appears to be PrP\textsuperscript{C}-independent while the late-onset progressive disorder is PrP\textsuperscript{C}-dependent.

**Role of PrP\textsuperscript{C} in Seizures**

Patients with AD have an increased incidence of unprovoked seizures.\textsuperscript{34,35} Epileptiform EEG discharges, including spikes and sharp waves in temporal lobe epilepsy patients can generate AD-like memory dysfunction.\textsuperscript{36} Accordingly, transgenic AD model mice such as J20 and APP\textsuperscript{swe}/PS1 ΔE9 mice exhibit altered network activity and epileptiform discharges.\textsuperscript{22,37} Interestingly, the APP/PS1 transgenic mice lacking PrP\textsuperscript{C} does not exhibit a convulsive seizure phenotype, which suggests that this electrographic abnormality of AD model mice requires PrP\textsuperscript{C}.\textsuperscript{22}

**Fyn Kinase in AD**

The discovery of A\textsubscript{B} oligomer binding to PrP\textsuperscript{C} in AD pathogenesis implies a neuronal signaling pathway downstream of the A\textsubscript{B}-PrP\textsuperscript{C} complex. Our recent study published shows that
Fyn kinase functions to mediate signal transduction from Aβ-PrPC complexes. We found that Aβ-PrPC activates Fyn pathway, and subsequently dysregulates NMDA receptor function.

Fyn is a member of the Src family of intracellular non-receptor tyrosine kinases family. These are nine members of Src family kinases. Five of them (Src, Fyn, Lck, Lyn and Yes) are expressed in central nervous system, but Src and Fyn are most highly expressed in the brain. Fyn activity, like that of other Src family kinases, is regulated by intramolecular interactions that depend on an equilibrium between tyrosine phosphorylation and dephosphorylation. In the basal state, catalytic activity is constrained by intramolecular interactions, such as engagement of the SH2 domain by a phosphorylated C-terminal tyrosine 527. Disruption of these interactions by phosphorylation at Tyr 416 in the activation loop of the kinase domain and/or by dephosphorylation of Tyr 527 results in Fyn activation.

Src family kinases have been implicated in neurodegenerative disease, including AD. In one study, PP2, a selective inhibitor of Src family kinases, was shown to be neuroprotective against Aβ. For AD transgenic models, the toxic effect of Aβ is blocked by the genetic ablation of Fyn, whereas overexpression of Fyn enhances Aβ-induced toxicity and AD-related phenotypes. Fyn regulates NMDA receptor trafficking and synaptic plasticity, and Fyn interacts with Tau to modulate phenotypes in AD mouse models.

There are multiple lines of evidence linking Fyn kinase to PrPC. Fyn is localized to lipid rafts as is PrPC, and clustering of PrPC activates Fyn kinase in certain cell lines. Fyn mediates mutant PrPC phenotypes in fish and worms. Based on these reports, we focused on Fyn as a candidate transducer of Aβ-PrPC signaling.

**Fyn Activation by Aβ-PrPC**

We found that Fyn kinase is specifically activated in response to synthetic Aβ oligomer in cell lines overexpressing PrPC (Fig. 1). In addition, Aβ oligomer-induced Fyn activation is observed in wild type cortical neurons, but not in Prnp−/− neurons. More importantly, AD brain extract containing PrPC-interacting Aβ species stimulates neuronal Fyn activation, but age-matched control brain extracts do not. The ability of AD brain extract to induce Fyn activation is absent from Prnp−/− neurons. Thus, AD-relevant preparations of Aβ oligomers activate neuronal Fyn via PrPC.

Fyn is known to phosphorylate the NMDA receptor subunit, NR2B. This phosphorylation regulates NMDA receptor trafficking and synaptic plasticity. NR2B phosphorylation and subsequent cell surface level of NR2B are enhanced during first 15 min of Aβ oligomer exposure but after 1–3 h, phosphorylation is suppressed and surface level of NR2B returns to the basal level. The biphasic effect of Aβ oligomer through PrPC/Fyn on surface NR2B is paralleled by changes in NMDA-induced calcium signaling. The NMDA receptor dysregulation induced by Aβ is eliminated in Prnp−/− and Fyn−/− neurons. Furthermore, we found that the Fyn activation by Aβ-PrPC complexes induces excitotoxicity and destabilizes dendritic spines. Aβ oligomer-induced cell death has now been shown to be PrPC-dependent.

Our studies define an Aβ oligomer signal transduction pathway that requires PrPC and Fyn to alter synaptic function, and that is likely to be directly relevant to AD. These observations pose the issue of how PrPC might be connected to Fyn kinase, since two proteins are localized in the opposite sides of the plasma membrane. One or more transmembrane proteins might serve as a co-receptor(s) for Aβ oligomer to couple PrPC with Fyn. It will be of interest to examine whether other Aβ signaling pathways, such as calcineurin, insulin receptors, autophagy, and Tau-directed kinases, are also PrPC-dependent. Investigation of the role of Aβ-PrPC-Fyn pathway in an even broader set of preclinical AD models will further define its relevance for human AD pathophysiology.

**Conclusion**

In summary, we have reviewed a series of studies showing that Aβ oligomer binding to PrPC mediates deleterious effects on neurons. Both well-characterized synthetic Aβ oligomers and human TBS-soluble AD extracts impair neuronal synapse function in a PrPC-dependent manner. The role of the Aβ/PrPC complex in AD pathophysiology is further supported by the
identification of a downstream Fyn pathway linking Aβ-PH in to NMDA receptor and Tau dysfunction. Being distinct from regulating the levels of Aβ, the PrPβ-Fyn pathway represents a novel target for AD therapy. This pathway is based specifically on neuronal toxicity of Aβ oligomers, and provides a link between Aβ and Tau pathologies in the disease. Of importance, the limited or absent phenotype of Prnpβ between Aβ and toxic amyloid Fβ. Chenn S, Pasternak JF, Kuo H, Ristic H, Lambert MP, Falsig J, et al. Prion protein and Abeta-amyloid oligomer interactions mainly with insoluble prion protein. J Biol Chem 2010; 286:15095-105; PMID:20702360; http://dx.doi.org/10.1074/jbc.M110.199356.


