Modeling Alzheimer's Disease: The Artifact of APP and Presenilin Overexpression

Frank Lee
eMail: frank@geon.us
Website: http://www.geon.us

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Abstract

In the transgenic mouse model that overexpresses amyloid precursor protein (APP) and presenilin (PS), both amyloid beta peptide (Aβ) and APP intracellular domain (AICD) are elevated. The pathological features, such as hyperexcitability and synaptic dysfunction, were commonly attributed to Aβ, but recent studies suggest that AICD could also make an important contribution. Furthermore, mice overexpressing APP/PS failed to recapitulate a hallmark of Alzheimer's disease: neurofibrillary tangles (NFT). Recently, this feature has been reproduced by a new modeling system called 3D cell culture. It turns out that the key event that enables 3D cell culture to produce robust NFT is elevated 4-repeat (4R) Tau (D'Avanzo et al., 2015). This finding supports the hypothesis that Tau pathology is caused mainly by Tau-mediated hyperexcitability (Paper 6), resulting from elevated 4R Tau (Paper 2).

Introduction

In a recent paper entitled "Calpain Activation in Alzheimer's Model Mice Is an Artifact of APP and Presenilin Overexpression", the authors called for revaluation of over 3000 publications based on overexpression of amyloid precursor protein (APP) and/or presenilin (PS) (Saito et al., 2016). Calpain can stimulate GSK-3 and CDK5 to phosphorylate Tau proteins (Paper 6). Hyperactive calpain, which may lead to Tau hyperphosphorylation and aggregation, is regarded as a critical step in the progression of Alzheimer's disease (AD) (Kurbatskaya et al., 2016). In mice overexpressing APP/PS, calpain is activated, together with the production of massive amyloid beta peptides (Aβ) (Liang et al., 2010). This led many researchers to believe that Aβ has the capacity to activate calpain (Tu et al., 2014). However, by using

APP knockin, which introduces pathogenic mutations without overexpressing APP, Saito et al. did not find evidence for the activation of calpain, despite massive Aβ accumulation.

Aβ is generated from APP via two consecutive cleavages. Cleavage of APP by the β-secretase creates sAPPβ and CTFβ. Subsequent cleavage of CTFβ by the γ-secretase generates Aβ and APP intracellular domain (AICD) (Paper 7). In the past three decades, most studies focused on Aβ, but recently AICD has received growing interest (Ghosal et al., 2009; Vogt et al., 2011; Lauritzen et al., 2012; Ghosal et al., 2016). This paper will examine previous publications based on APP/PS overexpression and show that the pathological features, such as hyperexcitability and synaptic dysfunction, that were attributed to Aβ could also be contributed by AICD. Furthermore, the latest finding that the 3D cell culture model (Choi et al., 2014) is able to recapitulate neurofibrillary tangles (NFT) actually supports the Microtubule Model presented in Paper 2 and Paper 6.

AICD/GSK-3 Signaling

Compelling evidence indicates that AICD promotes GSK-3 activity (Kim et al., 2003; Ryan and Pimplikar, 2005; Zhou et al., 2012; Ghosal et al., 2009), but its underlying mechanism is not clear. AICD has been demonstrated to be a transcription activator (Pardossi-Piquard and Checler, 2012). *In vitro* studies did reveal increased GSK-3 expression by AICD (Kim et al., 2003; von Rotz et al., 2004). However, the *in vivo* study did not exhibit elevated GSK-3 mRNA level (Ryan and Pimplikar, 2005). Another study found that AICD was able to associate with GSK-3 and directly enhance its activity (Zhou et al., 2012). It is also possible that AICD, together with its partner Fe65, may upregulate miR-342-5p to suppress Ankyrin-G expression, thereby resulting in hyperexcitability (Paper 7). The hyperactive neurons then cause Ca^{2+} overload to activate calpain, leading to GSK-3 activation (Paper 6).

Regardless of the underlying mechanism, the elevated GSK-3 activity by AICD may lead to hyperexcitability, synaptic dysfunction and Tau hyperphosphorylation (Figure 1).

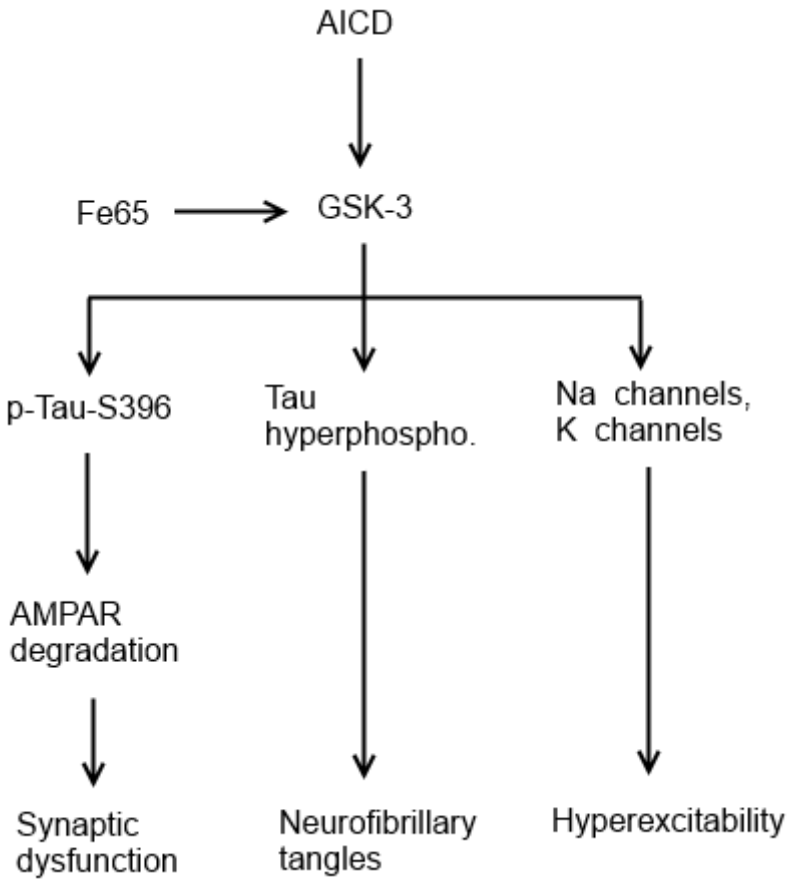

Figure 1. The effects of AICD/GSK-3 signaling.

Hyperexcitability

Hyperexcitability is a well documented feature of APP/PS overexpression (Palop et al., 2007; Minkeviciene et al., 2009; Busche et al., 2012; Xu et al., 2015; Bezzina et al., 2015). It was commonly attributed to the effects of elevated Aβ resulting from APP/PS overexpression. However, the APP/PS overexpression also increases AICD which may enhance excitability via Ankryin-G and GSK-3 (Paper 7). Indeed, mice overexpressing AICD and its binding partner Fe65 exhibit hyperactive GSK-3β (Ryan and Pimplikar, 2005) and seizure susceptibility (Vogt et al., 2011). Interestingly, a mutation within AICD (D664A) prevents these abnormalities despite high level of Aβ. Although D664 is known to be a caspase cleavage site (Deyts et al., 2016), this mutation could disrupt binding with an essential partner, rendering AICD inactive (Vogt et al., 2011).

Synaptic Dysfunction

Synaptic dysfunction is another feature of APP/PS overexpression. It is also a hallmark of Aβ-induced pathology. Therefore, the synaptic dysfunction observed in APP/PS overexpression was thought to arise from excess Aβ (Spires-Jones and Knafo, 2012). As pointed out in Paper 7, Tau phosphorylation at Ser396 is crucial for inducing AMPAR degradation - a major synaptic dysfunction. GSK-3β is the only kinase that may phosphorylate Tau at Ser396 without requiring other kinases (Wang et al., 2007). AICD is expected to influence synaptic plasticity because it can promote GSK-3 activity. Consistent with this notion, in APP transgenic mice, the D664A mutation (within AICD) has been demonstrated to maintain normal synaptic transmission, synaptic plasticity, and learning despite the presence of elevated levels of APP, Aβ, and even plaque accumulation (Saganich et al., 2006; Galvan et al., 2006).

Neurofibrillary Tangles

The neurofibrillary tangle (NFT) composed of hyperphosphorylated Tau is a hallmark of AD. Among all protein kinases that may phosphorylate Tau proteins, GSK-3β targets the largest number (~20) of phosphorylation sites (Wang et al., 2007). Extracellular Aβ does not stimulate GSK-3β activity (Zempel et al., 2010). Only the internalized Aβ may enhance GSK-3β activity via activation of caspase (Paper 7). Mice overexpressing APP and/or PS exhibit increased Tau phosphorylation, but no NFT (D'Avanzo et al., 2015). By contrast, co-expression of both AICD and Fe65 is sufficient to produce Tau aggregation (Ghosal et al., 2009). Since APP/PS overexpression increases only the AICD level, not Fe65, these results suggest that elevated AICD alone may enhance GSK-3 activity, but not to the level required for tangle formation. Addition of Fe65 could dramatically increase GSK-3 activity to cause Tau aggregation.

The Amyloid Cascade Hypothesis (Selkoe and Hardy, 2016) asserts that AD is initiated by excess Aβ. According to this hypothesis, Aβ should be able to drive Tau alteration, such as NFT in AD. The failure of massive Aβ in transgenic mice to produce NFT prompted Aβ proponents to develop alternative modeling systems. A novel technology, called induced pluripotent stem cell (iPSC), was found to have great potential in modeling AD (Yagi et

al., 2011). However, the original iPSC system still failed to recapitulate NFT. In the following years, researchers continued to improve the modeling systems. Recently, the 3D cell culture system has finally succeeded in producing robust NFT.

Why is 3D cell culture a better system for modeling AD? The answer lies in the 4-repeat Tau (D'Avanzo et al., 2015):

> More importantly, we found that 3D culture condition greatly elevated 4-repeat adult tau (4R tau) isoforms, which is essential for recapitulating tau pathology.

This finding is in good agreement with the hypothesis that Tau pathology is caused mainly by Tau-mediated hyperexcitability (Paper 6), resulting from elevated 4R Tau (Paper 2).