Unraveling the paradox of statins with human neurons: ~~uncovers~~ new leads in Alzheimer's disease

Joel W. Blanchard[1,2] and Li-Huei Tsai[1,2,3]*

1. Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

2. Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

3. Broad Institute of Harvard and MIT, Cambridge, MA 02139, USA

* To whom correspondence should be addressed: lhtsai@mit.edu

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Abstract

~~For decades, C~~conflicting clinical studies have ~~alternatively~~ reported that statins both reduce and accelerate cognitive impairments in Alzheimer's disease (AD). In this issue ~~of *Cell Stem Cell*~~, Van der Kant et al. (2019) use iPSC-derived neurons to thoroughly dissect the link between cholesterol synthesis, phospho-Tau, and amyloid-β, revealing new therapeutic opportunities in Alzheimer's disease and related dementias.

Main Text

Alzheimer's disease (AD), the most common form of dementia, is characterized by the pathological accumulation of phosphorylated Tau (pTau) and amyloid-β (Aβ) fragments in the brain. With no effective treatment, the number of patients diagnosed with AD is predicted to swell to over 13 million in the US and over 131 million worldwide by the year 2050(Hebert et al., 2013). Genetic studies in the 1990's suggested that Aβ was the main causative factor in AD because mutation or duplication in *APP*, the gene that encodes amyloid-β precursor protein, and in the genes *PSEN1* and *PSEN2* which encode Aβ processing components, lead to the familial forms of early onset AD(Canter et al., 2016). These seminal studies laid the foundation for the amyloid cascade hypothesis, which postulates that accumulation of Aβ in the brain initiates the pathophysiology of AD, leading to pTau accumulation and cognitive deficits~~(Hardy and Higgins, 1992.)~~. A main prediction of the amyloid cascade hypothesis was that therapeutics that block, prevent, or clear the accumulation of Aβ from the brain would also prevent~~,~~ pTau formation~~,~~ and cognitive decline. As a result, numerous effective strategies have been developed that clear and/or prevent the formation of Aβ plaques. Despite the ability to clear Aβ, no clinical studies to date have demonstrated that anti-amyloid therapies slow down cognitive degeneration in AD patients.

The inability of anti-amyloid therapies to stall cognitive decline has led to the idea that targeting pTau or both pTau and Aβ may be necessary for therapeutic efficacy in AD. Indeed, several studies have found that pTau levels are stronger predictors of cognitive outcomes compared to Aβ levels(Bejanin et al., 2017). Furthermore, the lack of correlation between Aβ and pTau suggests that pTau may not be downstream of Aβ as the amyloid cascade hypothesis predicts. Instead, pTau pathology in AD may develop at least in part through Aβ independent processes. Thus, understanding the mechanisms underlying pTau accumulation in the human brain and identifying strategies to prevent its build up could uncover promising new therapeutic opportunities for treating AD and Tau-related diseases.

In this issue, Van der Kant et al. (2019) examine the mechanism of pTau accumulation using iPSC-derived neurons from familial AD patients previously described to have elevated pTau and Aβ levels(Israel et al., 2012). Initially, they perform a small molecule screen of 1684 compounds assessing their ability to reduce the ratio of pTau (T231) to total Tau. From the primary screen, Van der Kant confirmed that 42 (2.49%) compounds reduce pTau–levels in fAD neurons. Encouragingly, six of these hits had previously been shown to suppress pTau-levels through interaction with microtubules, thus validating the screen. Interestingly, four of the 42 hits were statins, known inhibitors of cholesterol biosynthesis.

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Cholesterol metabolism has been extensively linked to AD pathogenesis(Di Paolo and Kim, 2011). The use of statins for the prevention and treatment of dementia received much attention after two epidemiological studies reported lower rates of dementia in statin users compared to non-users(Jick et al., 2000; Wolozin et al., 2000). Since these initial findings, numerous additional studies and meta-analyses have examined the effect of statins on AD and dementia, producing conflicting results with some studies finding that statins are beneficial while others report no therapeutic benefits or even acute cognitive impairments in patient receiving such treatments(Schultz et al., 2018). Mechanistically, there has been little insight into this paradox of statins. A limited number of mouse studies have reported that statins can reduce pTau *in vivo*(Boimel et al., 2009). However, the underlying mechanisms and whether this effect extends to human neurons remain unknown. In the absence of information on how statins affect human patient neuronal cells and influence human AD pathophysiology, the efficacy of using statins or targeting cholesterol synthesis as a therapeutic strategy for AD and dementia remains controversial.

Van der Kant and colleagues charge into this divisive void. Using chemical and genetic approaches, they mechanistically dissect the link between the cholesterol synthetic pathway and pTau levels in human AD patient-derived neurons. They find that inhibition of cholesterol synthesis, but not the opposing isopreniod pathway, significantly reduces pTau levels. This careful dissection pinpoints that cholesterol-esters, the main storage unit of intracellular cholesterol, are responsible for increasing pTau in human neurons rather than free cholesterol or other byproducts of cholesterol synthesis.

Corroborating ~~As~~ previous studies ~~have shown~~, Van der Kant et al. also observe that statins reduce the secretion of Aβ. The concurrent reduction of both pTau and Aβ again implies that pTau could be downstream of Aβ as predicted by the amyloid cascade hypothesis. However, Van der Kant et al. demonstrate that statins can even reduce pTau in *APP*-deficient neurons, suggesting the mechanism is independent of the *APP* gene or its product Aβ. They further confirm this by using CRISPR/Cas9 mutagenesis to delete a recently identified cholesterol binding domain~~s~~ in *APP*.~~(Barrett et al., 2012).~~ Mutating APP's cholesterol binding domain abolished statin's ability to reduce Aβ secretion, while retaining statin~~'s~~' ability to reduce pTau levels~~. Thus~~, thus suggesting that cholesterol-mediated pTau formation is independent of cholesterol-mediated Aβ secretion.

Van der Kant et al. next examine how cholesterol-esters influence pTau levels. They find that cholesterol-esters do not affect major kinases such as GSKβ that are known to phosphorylate Tau. However, they find that reduced cholesterol-esters increase overall proteosomal activity, which in turn leads to enhanced degradation of pTau and total Tau. These results offer intriguing insight into how statins could be regulating pTau levels and suggest that targeting proteosomal activity by modulating cholesterol biosynthesis may be an effective strategy to reduce pTau levels.

The mechanistic insight from human neurons implies that statins offer a potential therapeutic strategy to simultaneously reduce both pTau accumulation and Aβ secretion. However, when Van der Kant and colleagues apply statins to astrocytes even at low concentrations, they are cytotoxic. These dichotomous observations, that statins can clear pTau in neurons~~,~~ but also cause cytotoxicity in astrocytes, perhaps provide some perspective into the paradoxical effects of statins reported in clinical trials. Fortunately, the detailed mechanistic dissection in this study revealed alternative opportunities for reducing pTau and Aβ through modulating cholesterol biosynthesis. They find that activating

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CYP46A1 with Efavirenz promotes the conversion of cholesterol to 24(S) hydroxycholesterol reducing cholesterol-esters, which leads to a reduction in pTau and Aβ secretion and does not induce astrocyte death.

Whether CYP46A1 is a viable therapeutic target for AD and dementia largely remains to be determined. Future studies are needed to determine the effect that modulating CYP46A1 has on other cells and tissues in the brain such as oligodendrocytes, microglia, and the blood-brain barrier. Additionally, animal and clinical studies are needed to establish whether activating CYP46A1 *in vivo* can safely reduce pTau and Aβ and prevent cognitive degeneration. However, through their careful mechanistic dissection and the biological insight gained, Van der Kant and colleagues clearly illustrate the value of using human iPSC-derived cells for screening and mechanistic studies.

Figure Legend.

Van der Kant and colleagues find that statins reduce the levels of cholesterol-esters, which leads to a reduction in the pathogenic proteins pTau and Aβ produced by neurons. However even at low concentrations statins are toxic to astrocytes. Fortunately, their mechanistic dissection pinpoints that manipulating CYP46A1 a downstream component of the cholesterol biosynthesis can also reduces pTau and Aβ, while avoiding the cytotoxic effects on astrocytes.

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