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Amyloid β Clearance Is Disrupted by Depletion of Low-Density Lipoprotein Receptor-Related Protein 4 (LRP4) in Astrocytes

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Review of Zhang et al.

Alzheimer's disease (AD) is the most common type of dementia, a syndrome characterized by progressive loss of cognitive function that affects ~50 million people worldwide (Nichols et al., 2019). Given its high prevalence and the severity of its consequences, this pathology has become an area of intense research to elucidate its underlying molecular bases and to design therapeutic strategies. One of the main hypotheses regarding the development and progression of the disease is the amyloid cascade hypothesis, which postulates that dysregulation of biosynthesis, increased aggregation, and/or decreased clearance of amyloid β (Aβ) peptides are the main drivers of AD. Consistent with this hypothesis, extracellular accumulation of Aβ leads to the formation of amyloid aggregates, which have neurotoxic effects including the alteration of neuronal morphology and synapses (Selkoe and Hardy, 2016).

In recent years, the contribution of astrocytes to AD has been studied. Astrocytes are one of the most abundant cell types in the adult mammalian brain, and in AD they become activated and cluster in close proximity to amyloid plaques (Itagaki et al., 1989). The role of astrocytes in the progression of AD remains a matter of debate, however: they have been described as both neuroprotective and neurotoxic (Perez-Nievas and Serrano-Pozo, 2018). One of the main neuroprotective roles attributed to astrocytes in AD is the clearance of Aβ aggregates from the extracellular milieu.

The process of Aβ clearance depends on apolipoprotein E (ApoE) and receptors of the low-density lipoprotein (LDL) receptor family (LDL-R). The main physiological function of ApoE is to transport lipids and cholesterol throughout the body, and LDL-R members mediate the endocytosis of the lipoprotein particles into cells (Liao et al., 2017). Importantly, ApoE has been found to interact with Aβ and affect its aggregation and clearance in AD (Castellano et al., 2011; Wisniewski and Drummond, 2020). Moreover, the ε4 isoform of human ApoE is the strongest genetic risk factor for the development of late-onset AD, and possession of this allele is associated with a decrease in Aβ aggregate clearance in aged mice (Castellano et al., 2011). Previous studies have shown that the LDL-R family receptor LRP1 is critical for Aβ uptake and degradation by astrocytes (Liu et al., 2017). In a recent report in *The Journal of Neuroscience*, Zhang et al. (2020) provide evidence that another member of the LDL-R family, low-density lipoprotein receptor-related protein 4 (LRP4), may also be relevant in AD pathogenesis.

First, the authors showed that in primary cultures of astrocytes from wild-type mice, *Lrp4* was the most abundantly expressed member of the LDL-R family. They then showed that postmortem human brain samples from AD patients exhibited lower LRP4 abundance in the hippocampus and cortex than healthy controls. Furthermore, selective ablation of Lrp4 from astrocytes increased Aβ deposition in the cortex and hippocampus in a mouse model of AD (5xFAD), whereas depleting LRP4 from neurons had no effect. Together, these data suggest an association between astrocyte expression of LRP4 and extracellular accumulation of Aβ in the brain.

Next, Zhang et al. (2020) asked whether LRP4 is involved in synaptic and cognitive function. To evaluate this, they ablated Lrp4 expression in neurons and astrocytes of 5xFAD and control mice. Electrophysiological recordings in hippocampal slices revealed that the frequency of miniature EPSCs and the ability to induce long-term potentiation were reduced in Lrp4-deficient 5xFAD mice compared with control 5xFAD mice. In addition, dendritic spine loss in the hippocampus and impairments in working and spatial memory were

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greater in Lrp4-deficient 5xFAD mice than in control 5xFAD mice. Finally, the authors evaluated local field potentials in the ventral hippocampal–prefrontal cortex circuitry, which is relevant for modulating working memory. Of note, in 5xFAD mice this circuitry exhibited a decrease in coherence at theta, beta, and gamma frequencies relative to control mice, and this effect was amplified by LRP4 ablation. Together, these findings suggest that LRP4 contributes to impairments in synaptic plasticity, memory, and hippocampal–prefrontal cortex connectivity in AD.

As noted above, LDL receptors interact with ApoE and Aβ (Castellano et al., 2011; Wisniewski and Drummond, 2020). Other work has shown that LRP4 and ApoE interact in adult rat brain homogenates (Lu et al., 2007) and that the AD-linked ApoE ε4 isoform decreases the rate of Aβ uptake in N2a cells (Li et al., 2012) and clearance from the brain interstitial fluid in aged AD model mice (Castellano et al., 2011). Therefore, Zhang et al. (2020) evaluated the interaction between LRP4 and different alleles of human ApoE expressed in astrocyte cell lines. First, they observed that LRP4 ablation in cultured astrocytes decreased Aβ uptake and increased Aβ levels in the extracellular medium. Then, they found that LRP4 binds to ApoE3, suggesting that the LRP4/ApoE complex may induce Aβ clearance. Moreover, they found that the affinity of LRP4 for ApoE-ε4 was lower than its affinity for other ApoE isoforms. This might explain the increased amyloid accumulation in patients carrying the ε4 allele, as a decrease in ApoE–Lrp4 affinity would impair Aβ clearance.

Zhang et al. (2020) show that the ablation of astrocytic Lrp4 increases brain amyloid burden by impairing astrocytic Aβ uptake without affecting Aβ production. This is consistent with previous studies, which have shown that global LDL-R knockout (Katsouri and Georgopoulos, 2011) and astrocyte-specific Lrp1 ablation (Liu et al., 2017) increase amyloid pathology in AD mice by reducing Aβ uptake and clearance, without affecting the cleavage of amyloid precursor protein. Conversely, LDL-R overexpression in neurons and astrocytes enhanced brain-to-blood clearance of Aβ in the PDAPP mouse model of AD, and this reduced brain Aβ plaque load and ApoE concentration (Castellano et al., 2012). Together, this evidence points to a shared contribution of different members of the LDL-R family to the process of Aβ uptake and clearance by astrocytes.

In addition to taking up Aβ associated with ApoE, LRP4 might contribute to Aβ clearance and AD pathogenesis through the modulation of purinergic signaling in the hippocampus. Astrocyte-specific LRP4 knock-out mice show reduced glutamate neurotransmission and impaired locomotor activity and spatial memory associated with an increase in ATP release in the hippocampus (Sun et al., 2016). Aβ also induced an increase in extracellular levels of ATP in hippocampal slides, leading to neuronal death. Conversely, suppressing purinergic signaling reduced Aβ accumulation and impairments in synaptic and cognitive function seen in AD (Erb et al., 2019). Furthermore, genetic inhibition of purinergic receptors enhances Aβ phagocytosis mediated by microglia and contributes to its clearance (Ni et al., 2013). Collectively, these studies suggest that LRP4 deficiency in hippocampal astrocytes enhances purinergic signaling, impairing Aβ clearance and contributing to AD pathogenesis.

Zhang et al. (2020) showed that brain samples from AD patients had reduced LRP4 protein levels, but a previous study showed that astrocytes from aged mice present an upregulation of *Lrp4* (Clarke et al., 2018), suggesting that LRP4 expression is controlled through post-translational mechanisms in the context of AD. LRP4, as an endocytic receptor, is intimately linked to the endolysosomal system, whose functioning in astrocytes is abnormal in AD. A recent study showed that astrocytes exposed to the 42-residue isoform (Aβ42) have enlarged early endosomes, which might be a consequence of lysosomal dysfunction (Söllvander et al., 2016). ApoE ε4 expression in astrocytes has been shown to sequester LRP1 in acidified endosomes and to impair Aβ clearance (Prasad and Rao, 2018). The effects of AD-related endolysosomal dysfunction on surface and total level of astrocytic LRP4 expression have yet to be elucidated, but this may contribute to decreased function of this receptor. Another post-translational cellular mechanism involved in the control of LRP4 abundance is degradation through the ubiquitin–proteasome system. Muscles of aged mice exhibited greater ubiquitination and proteasome-mediated degradation of Lrp4 than those of 3-month-old mice, and exogenous expression of α-sarcoglycan stabilized LRP4 and decreased neuromuscular junction aging (Zhao et al., 2018). α-Sarcoglycan is not expressed in the brain, but perhaps other components of the extracellular matrix present in the brain contribute to LRP4 stability in astrocytes. Further studies are needed to determine the post-translational mechanisms that reduce LRP4 levels in astrocytes in AD and also the potential stabilizers of the receptor that could serve as therapeutic targets.

In conclusion, Zhang et al. (2020) provide strong evidence that astrocytic LRP4 plays a key role in Aβ clearance and AD pathogenesis. Although the details of the *in vivo* mechanism underlying these effects remain to be elucidated, it likely involves a reduction in the ApoE–LRP4 interaction and alterations in purinergic signaling in the hippocampus. Further studies are needed to dissect the contribution of astrocytic LRP4 to AD and to explore potential therapeutic approaches related to LRP4 function.

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