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Previews

Neuronal γ-secretase regulates synaptic functions via cholesterol homeostasis

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In this issue of *Neuron*, Essayan-Perez and Südhof[1] demonstrate roles for γ-secretase in the regulation of synaptic functions in human neurons. Chronic attenuation of γ-secretase activity increases synapse formation but decreases neurotransmission (i.e., the probability of presynaptic release), likely due to impairment of cholesterol metabolism.

Alzheimer's disease (AD) is characterized by progressive deterioration of memory and cognitive functions. One of the major neuropathological changes that contributes to such deficits is the failure of synaptic functions. Amyloid-beta (Aβ) peptides, particularly $A\beta_{42}$, are toxic peptides that accumulate abnormally in the brains of AD patients. Aβ detrimentally affects synaptic functions by disrupting neurotransmitter release and reuptake, impairing synaptic transmission and interfering with proper synaptic connectivity.[2,3]

The major genetic risk factors of familial AD are the *APP*, *PSEN1*, and *PSEN2* genes. *APP* encodes the amyloid precursor protein (APP), the precursor of Aβ. Meanwhile, *PSEN1* and *PSEN2* encode the presenilin 1 (PS-1) and presenilin 2 (PS-2) isoforms, respectively, which are the catalytic subunits of the γ-secretase cleavage enzyme. γ-secretase is responsible for the proteolysis of various membrane proteins, including APP. Notably, the pathological Aβ peptides in AD are generated through γ-secretase-dependent cleavage of APP via the amyloidogenic pathway. In this pathway, APP is first cleaved by β-secretase to generate C-terminal fragments and secreted APPβ. Subsequently, the C-terminal fragments are cleaved by γ-secretase to generate Aβ peptides and APP intracellular domain. Transgenic mouse models of AD, such as APP/PS1 and 5XFAD, which carry mutations in *PSEN1* and *APP*, exhibit early synaptic abnormalities.[4] Furthermore, more than 100 γ-secretase substrates, including Notch, Eph, and Neurexin receptors, can be cleaved by γ-secretase and are involved in the regulation of synaptogenesis and synaptic functions.[5] However, the roles of γ-secretase in synaptic functions have not been extensively explored.

Here, Essayan-Perez and Südhof demonstrate the role of neuronal γ-secretase in regulating synaptic functions by modulating cholesterol homeostasis (Figure 1).[1] To investigate this, the authors utilized a system consisting of induced human neurons (iNs) derived from embryonic stem cells co-cultured with mouse glial cells. They generated a co-culture system with chronically reduced γ-secretase activity through two methods: (1) treating iNs with γ-secretase inhibitors or (2) suppressing γ-secretase activity in iNs by knocking out *PSEN1* in embryonic stem cells prior to their induction into iNs. Suppressing γ-secretase activity using these approaches results in impaired synapse formation and synaptic transmission.

The authors found that chronic attenuation of γ-secretase activity led to an increase in synapsin (a presynaptic protein) and PSD-95 (a postsynaptic protein) as well as their colocalized puncta in neurons, suggesting enhanced excitatory synapse formation. However, this increase did not correspond to a rise in basal synaptic transmission, evidenced by no change in the frequency of miniature excitatory postsynaptic currents (mEPSCs). Despite this, the neurons with decreased γ-secretase activity exhibited decreased strength and magnitude of synaptic transmission in terms of amplitude and charge transfer of evoked excitatory postsynaptic currents (eEPSCs). Further analysis revealed an increase in paired-pulse depression, suggesting a deficit in neurotransmitter release probability. These findings collectively indicate that neurons with decreased γ-secretase activity exhibit differential dysregulation in both structural synaptic changes and functional synaptic activity.

Subsequent bulk transcriptome analysis of the human neuron-mouse glia co-culture upon administration of γ-secretase inhibitor revealed that the differentially regulated genes were primarily from human neurons. This suggests that the impaired neurotransmitter release, induced by decreased γ-secretase activity, is mainly mediated through the regulation of neuronal molecular and cellular responses. Intriguingly, most differentially upregulated neuronal genes are associated with cholesterol synthesis and transport, indicating an alteration in cholesterol metabolism in γ-secretase-suppressed

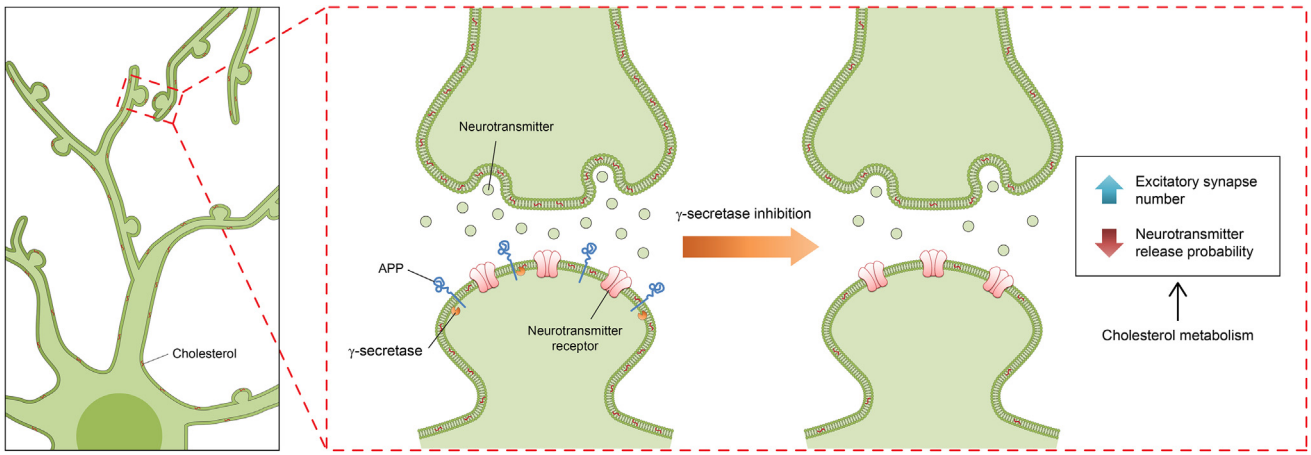

Figure 1. γ-secretase regulation of synaptic functions via cholesterol metabolism
Human neurons, derived from embryonic stem cells, exhibit enhanced excitatory synapse formation and attenuated synaptic transmission when γ-secretase activity is chronically suppressed. The synaptic transmission deficit is attributed to deregulated cholesterol metabolism. APP, amyloid precursor protein.

neurons. Supporting this finding, neurons with decreased γ-secretase activity also exhibited lower intracellular cholesterol levels. Furthermore, treatment with inhibitors of HMG-CoA reductase, the critical enzyme involved in cholesterol synthesis, led to dysregulated cholesterol metabolism along with decreased neurotransmitter release in iNs. This was evidenced by increased expression of genes associated with cholesterol synthesis as well as decreased synaptic transmission.

Cholesterol is a major lipid component that is essential for maintaining the integrity and fluidity of cell membranes. In the brain, cholesterol is highly enriched at synapses and is suggested to regulate various synaptic functions, including synaptic vesicle exocytosis in neurons.[6] Moreover, cholesterol homeostasis is tightly regulated via its synthesis, intracellular trafficking, and release. The present study has revealed an association between γ-secretase and cholesterol metabolism in neurons, particularly in the regulation of neurotransmitter release. Nevertheless, the molecular mechanisms by which the suppression of γ-secretase activity disturbs cholesterol metabolism remain unknown. A recent study shows that depleting APP in human iNs also results in deficits in synaptic vesicle release and recycling. Meanwhile, co-culturing these APP-depleted iNs with astrocytes or providing cholesterol supplementation rescues this impairment in synaptic vesicle cycling.[7] Although glial cells such as astrocytes and microglia are important for modulating synaptogenesis and synaptic functions, recent studies have highlighted their roles and dysregulation in synaptic degeneration during AD pathogenesis.[8,9] Although the gene expression analysis in the present study does not support the role or regulation of glial cells in the neuron-glia co-culture system's synaptic functions upon γ-secretase activity suppression, the involvement of glial cells should not be ruled out. Accordingly, it would be worthwhile to examine the changes in immune-related genes, owing to their importance in regulating synaptogenesis and synaptic functions.

One of the critical mediators of cholesterol metabolism and transport between glial cells and neurons is apolipoprotein (APOE). Importantly, the ApoE4 isoform, which is the strongest known risk factor for sporadic AD, impairs cholesterol metabolism and increases cholesterol level in neural cells.[10] Thus, the present study suggests the existence of interactions between amyloidosis pathways and cholesterol homeostasis in the regulation of neurotransmitter release and synaptic transmission. Therefore, further investigation into the mechanistic interplay between these pathways may provide valuable insights into the pathological mechanisms underlying synaptic deficits in AD pathogenesis. Additional analysis is needed to confirm whether there is a causal relationship between alterations in cholesterol metabolism and subsequent attenuation of synaptic transmission.

Given that the suppression of γ-secretase activity in the neuron-glia co-culture system is chronic, it is of interest to examine how this suppression modulates synaptic functions in neurons. Thus, it is important to identify the physiological substrates of γ-secretase in neurons, as it processes various membrane substrates[5] involved in essential cellular functions, including cell adhesion, immune response, phagocytosis, and gene transcription. Disruptions in these processes could impact synaptic formation and functions. Moreover, several γ-secretase substrates, such as lysosomal acid phosphatase 2 (ACP2), apolipoprotein E receptor 2 (ApoER2), low-density lipoprotein receptor (LDLR), and sortilin, are known to play critical roles in cholesterol homeostasis and functions. It is worthwhile to investigate whether these proteins serve as neuronal substrates for γ-secretase and are involved in cholesterol-dependent synaptic transmission. Understanding the homeostasis of these membrane substrates and their γ-secretase-mediated cleavage products in neurons may provide insights into the connection between γ-secretase and cholesterol in the regulation of synaptic connectivity and transmission.

Another factor that significantly affects synaptic functions is the presence of Aβ peptides. Aβ peptides have been shown to have detrimental effects on

synaptic functions, leading to synapse loss, impaired synaptic transmission (including neurotransmitter release), impaired synaptic plasticity, and synaptic hyperactivity.[2,3] Most *PSEN1* gene variants associated with familial AD are linked to increased production of $A\beta_{42}$. However, it remains controversial whether the gain or loss of PS-1 function contributes to the dysregulation of Aβ production, as increased Aβ production in familial AD can be a direct result of γ-secretase activity or an indirect effect of other γ-secretase substrates. To better understand how alterations in γ-secretase contribute to synaptic deficits in AD, investigating human iNs carrying AD-associated *PSEN1* variants may help uncover the association between γ-secretase and cholesterol metabolism in the regulation of synaptic functions. Such research efforts will advance our understanding of AD pathogenesis and aid in the development of novel therapeutic approaches.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Force opens a monomeric channel pore

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In this issue of *Neuron*, Zheng et al.[1] report the monomeric architecture of mechanosensitive ion channels TMEM63A and TMEM63B. They show that these high-threshold channels function as monomers on the plasma membrane.

Mechanical stimuli are vital environmental cues that can be perceived in a timely manner by living organisms in all kingdoms of life, ranging from bacteria to plants and humans. The known sensors for mechanical signals include diverse proteins, some of which are called mechanosensitive channels.[2] These channels are multi-pass transmembrane proteins that open their ion permeation pathways in response to mechanical stimuli. The fast response kinetics of mechanosensitive ion channels and their unique ability to convert mechanical signals to electrical signals make them the frontline detectors of mechanical stimuli.

To date, several classes of mechanosensitive channels have been identified, including MscS channels, MscL channels, NompC channels, Piezo channels, K2P channels, OSCA/TMEM63 channels, transmembrane channel-like (TMC) channels, and more. On one hand, extensive studies have established that these channels are involved in diverse but important physiological processes, ranging from drought response in plants to hearing sensation in humans. On the other hand, there is great interest in understanding how these proteins sense mechanical stimuli to open their highly regulated channel pores. This mechanistic knowledge will not only help us better understand the