Clusterin transduces Alzheimer-risk signals to amyloidogenesis

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Dear Editor,

Deposition of amyloid-β (Aβ) to form neuritic plaque (NP) is the hallmark of Alzheimer’s disease (AD). Major non-genetic risk factors such as ageing, stroke, diabetes and other conditions facilitate AD pathogenesis via unclear mechanisms. Furthermore, the mechanism underlying NP formation is unclear. Increasing Aβ causes NP in familial AD patients and in transgenic AD mice robustly expressing Aβ, but the NP formation requires long-term Aβ accumulation. Homogenates of AD brains seed NP nucleation in receiving brains, but the nature of the seeds and the endogenous seeds are unknown. Dysregulation of clusterin (CLU) has been implicated in AD pathogenesis. CLU gene contains several AD-associated intronic SNPs and its product, clusterin (CLU), is increased in the brain tissues, cerebrospinal fluid (CSF), and plasma of AD patients may have anti-amyloidogenic roles, but CLU knockout significantly reduces NP by unknown mechanism1 (Extended note 1).

CLU is a secretory protein mostly synthesized in astrocytes in the brain, but is highly inducible in neurons by AD risk factors. In the brains of 3-month wild-type mice, CLU was detected with specific antibodies (Supplementary Fig. S1a) only in neurons in brain stem (Supplementary Fig. S1b). In aged and type-2 diabetic (insulin-receptor inhibition) model mice, CLU extensively accumulates in cortical neurons (Fig. 1a, b). In stroke (middle cerebral artery occlusion, MCAO), lactic-acid-treated (mimicking acidosis, stroke, hemorrhage, and Herpes simplex virus-infected model mouse brains, CLU was upregulated in neurons and extracellularly in affected regions. Upon neuroinflammation induced by intracerebral-ventricle (ICV)-injected lipopolysaccharide, CLU upregulation was only extracellular (Supplementary Fig. S1c–h). Thus, all major AD risk factors converge at CLU upregulation. CLU localizes to all NPs in AD mice (Supplementary Fig. S1i), but no glial CLU was detectable under any condition.

Neuronal CLU is partly taken-up from extracellular space. Recombinant human CLU (rhCLU) appeared exclusively in cortical neurons upon ICV injection (Supplementary Fig. S2a). CLU is also endogenously expressed in primary neurons (PN). Several stress-inducers increased intracellular CLU, but only senescence by prolonged culturing upregulated both intracellular and extracellular CLU (Supplementary Fig. S2b, c). The extracellular increase was not simply due to accumulation over time, because medium CLU recovered overnight after complete medium change (Supplementary Fig. S2d). An CLU-shRNA driven by neuron-specific syn1 promoter decreased CLU in a time-dependent manner (Supplementary Fig. S2e), further indicating an ageing-dependent CLU production in neurons. Therefore, CLU proteasome in neurons is strictly regulated but changes during ageing. HDAC activator exifone and ATM kinase inhibitor Ku55933 inhibited CLU, suggesting epigenetics- and DNA damage-regulated CLU expression (Supplementary Fig. S2f, g).

To examine the effect of neuronal CLU upregulation on amyloidogenesis, 4.5-month APP/PS1ΔE9 mice were infected by AAV-PHP.EB expressing human CLU (hCLU) under the syn1 promoter by ICV injection. hCLU overexpressed under the astrocytic GFAP promoter in adult brains largely retained in astrocytes (Supplementary Fig. S3a), which is not a physiological/pathological condition. The overexpressed hCLU in neurons decreased the viability of APP/PS1ΔE9 mice (Fig. 1c), and aggravates depression/anxiety (Fig. 1d) that are common symptoms of AD affecting cognition. hCLU expression increased Aβ1–40, Aβ1-42, and the average size of thioloavin-S (ThioS)-stained NPs by 14.81 ± 6.248%, 24.29 ± 8.781%, and 18.46 ± 4.182%, respectively (Fig. 1e, Supplementary Fig. S3b). Neurons with highly overexpressed hCLU showed decreased or abolished NeuN with concomitant active-caspase-3 (Supplementary Fig. S3c, d), suggesting apoptosis. Additionally, the A1 astrocyte marker-complement-3 was upregulated in these mice (Supplementary Fig. S3e).

CLU enhances amyloidogenesis through multiple mechanisms. CLU at 1:1 ratio to Aβ1-42 abolished Aβ degradations by BACE2 and insulin-degrading enzyme (Fig. 1f, g). (Extended note 2). Moreover, CLU strongly binds to the juxtamembrane-helix (JH) of C99 (Supplementary Fig. S4a), and competes for a key γ-secretase component nicastrin binding to C99, which lifts γ-secretase inhibition by JH and results in lower C99 level and higher extracellular Aβ in PC12 cell and in the PN of APP transgenic mice (Supplementary Fig. S4b–d, Fig. 1h). γ-secretase inhibition abolished CLU’s effect on C99 in PC12, but increased C99 in CLU-overexpressing PN compared to FLAG-expressing PN (Fig. 1h), presumably because CLU activates C99 generation via BACE2 that is much higher in PN than in cell lines (Supplementary Fig. S4e).

CLU overexpression reduced intracellular Aβ in PN (Fig. 1h) likely by enhancing Aβ secretion, γ-cleavage of Notch was unaffected by CLU (Supplementary Fig. S4f).

CLU in wild-type PN culture resides in extracellular puncta (Fig. 1i) resistant to Triton-X100 (TX) extraction and stained by ThioS (Fig. 1j, k). These puncta co-stained with condensed DNA indicating apoptotic/necrotic cells. ThioS and CLU co-stained puncta were also observed in penumbra after stroke (Fig. 1l). Both rhCLU and synthetic Aβ1-42 added to PN attached to these puncta (Fig. 1m–p). Mass spectrometry analysis revealed that all the TX-insoluble proteins are NP-enriched proteins despite of the absence of Aβ (Supplementary Table S1). These Aβ-independent puncta are therefore dubbed “NP seeds”.

To facilitate apoptotic cell clearance and prevent autoimmunity, CLU targets to apoptotic neutrophils through binding to surface histones. Apoptosis or oncosis of wild-type PN markedly increased TX-insoluble histone-H3 (Supplementary Fig. S5). DNase-I treatment to expose histones increased TX-insoluble endogenous mouse CLU (mCLU) and TX-insoluble rhCLU (Fig. 1k, n), suggesting stronger CLU attachment. Upon the addition of Aβ1-42 to PN, DNase-I treatment increased TX-insoluble Aβ1-42 (iAβ) by 1.5–2-fold. Suppressing mCLU in PN reduced iAβ by 47.56 ± 5.114% and 54.02 ± 4.957% with or without DNase-I treatment, respectively. Overexpressed hCLU in PN barely affected iAβ possibly because of sufficient endogenous mCLU, but enhanced DNase-I-dependent iAβ by 94.11 ± 11.34% (Fig. 1p). Hence, CLU is indispensable for iAβ...
deposition and acts synergistically with DNase to increase iAβ at the sites of cell death. Similar DNase activity has been detected in CSFs of AD and healthy people (Fig. 1q). Given that regardless of its effects on Aβ level (Extended note 3), CLU is required for NP in vivo, dead/dying cells may efficiently sequester soluble Aβ through CLU to generate NP, and Aβ level may be unessential (Supplementary Fig. S6).

CLU in the serum, CSF, and the conditioned medium of PN shows different pattern in heparin-manganese fractionation to isolate lipoproteins (Supplementary Fig. S7a, b). In a transgenic
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AUTHOR CONTRIBUTIONS
Z.W., X.L., L.W., W.S., and Z.W. conceived and designed the experiments. X.L., R.C., W.S., and Y.W. wrote the paper. All authors reviewed the paper.

DATA AVAILABILITY
The raw data are available from the corresponding authors.

ADDITIONAL INFORMATION
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Competing interests: The authors declare no competing interests.
Ethics approval: This study was approved by the Ethics Committees of the Xuanwu Hospital of Capital Medical University (KY-Z-2020-135-02, GDREC2018338H [R1]) China, and was conducted in accordance with the principles stated in the Declaration of Helsinki. Animal experiments were carried out in strict accordance with the guidelines for the Care and Use of Laboratory Animals in preclinical research from the Zhengzhou University, Jining Medical University, and Beijing Friendship Hospital Ethics Committees. The animal protocol was approved by the Institutional Animal Care and Use Committees of Zhengzhou University, Jining Medical University, and Beijing Friendship Hospital.

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