Enhanced oxidative stress-regulated by cholesterol promotes necroptosis in Alzheimer's disease

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INTRODUCTION

Alzheimer's disease (AD) is characterized by extensive neuronal loss, however, how neurons die in the central nervous system still remains unclear. Caspases, primary effectors of apoptosis, are activated in AD brains but apoptotic morphology is not evident. Furthermore, the progress of the disease that lasts for decades seems incompatible with an apoptotic death program. A recent study offers compelling evidence of active necroptosis, a regulated inflammatory cell death, in post-mortem AD brains (**1**), but still, the events that trigger or regulate necroptosis in AD are not fully known. It has been reported that oxidative stress may be determinant as to whether a cell initiates necroptosis (**2**); it is, therefore, likely that mitochondrial dysfunction, observed in the early stages of AD, might favor cells to undergo necroptosis. Previous studies from our group have shown that high intracellular cholesterol levels deplete the mitochondrial pool of GSH, thus sensitizing neurons against amyloid-beta (Aβ)-induced mitochondrial oxidative stress (**3**). Therefore, **the present study is aimed to** evaluate whether cholesterol can regulate the necroptotic pathway

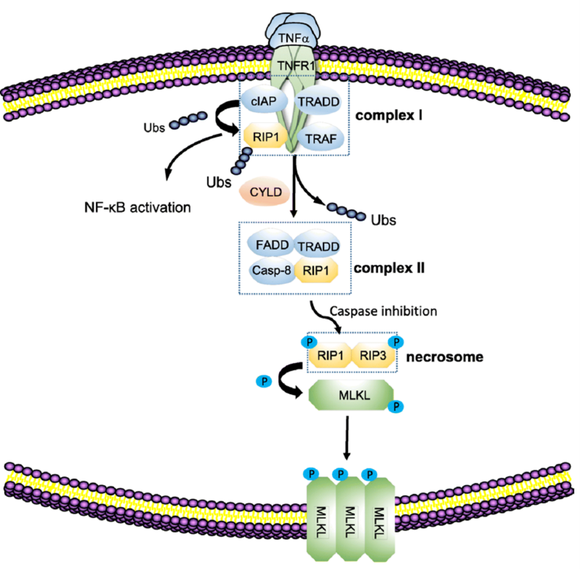
METHODS

APP-PSEN1-SREBP-2 transgenic mice were generated from crossbreeding of B6C3-Tg(APPswe,PSEN1dE9)85Dbo/J mice [express a chimeric mouse/human amyloid precursor protein (isoform 695) with the Swedish mutation (Mo/HuAPP695swe) and mutant human presenilin 1 (PSEN1dE9)] and B6;SJL-Tg(rPEPCKSREBF2)788Reh/J mice that overexpress the active form of the sterol regulatory element binding protein 2. These mice display increased total brain cholesterol levels and selective depletion of mitochondrial GSH levels (**4**).

The human neuroblastoma SH-SY5Y cell line was cholesterol-enriched with the soluble cholesterol:methyl-β-cyclodextrin complex (CHO:MCD, 50mg/ml). Necroptosis was induced using 10 ng/ml TNFα and 10mM qVD-Oph. In some cases, cells were pre-treated with RIPK1 inhibitor necrostatin (20mM), RIPK3 inhibitor GSK'872 (1mM) or glutathione ethyl ester (GSHee,1-2 mM).

RESULTS

Necroptosis signaling pathway



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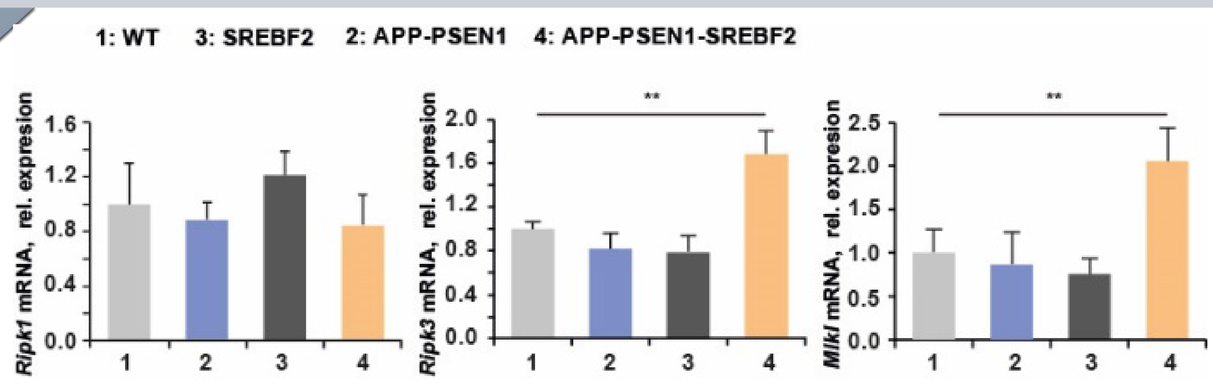
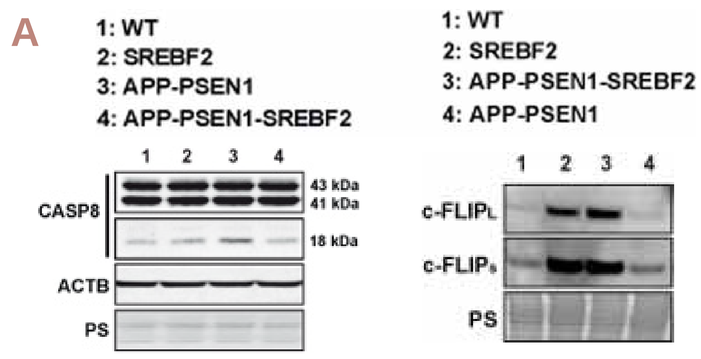


Up-regulated expression of necroptosis-related proteins in brains of APP-PSEN1-SREBF2 mice. mRNA expression levels of RIPK1, RIPK3 and MLKL in brain homogenates from WT and transgenic mice. Transcript copies were expressed as relative levels referred to the expression in WT mice (n = 3). **p<0.01.

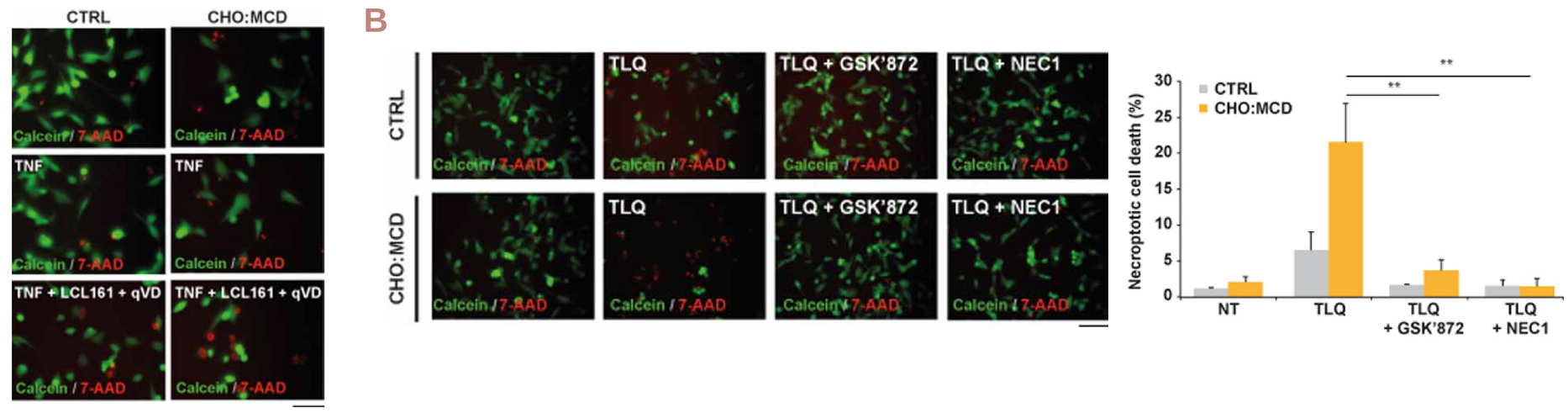
2

Increased caspase-8 inhibition that correlates with an enhanced necrosome assembly in APP-PSEN1-SREBP-2 mice. A) Western blot analysis of caspase-8 and c-FLIP in brain lysates of WT and transgenic mice. **B)** Sequential solubility analysis of RIPK1, RIPK3, and MLKL showing an increased presence of the necroptotic proteins in high insoluble fractions of brain lysates from APP-PSEN1-SREBF2 mice. F1: TBS soluble , F2: 1% Triton soluble, F3: 2% SDS soluble, F4: 8M urea soluble.



3

Cholesterol-enriched SH-SY5Y cells displays increased cell death inhibited by RIPK1 and RIPK3 inhibitors. A) Representative confocal images showing dying cells (7-AAD-stained cells) with intact nuclei when exposed to TNF plus LCL-161 and qVD-Oph. **B**) Representative confocal images showing the protective effect of necrostatin (RIPK1 inhibitor) and GSK'782 (RIPK3 inhibitor). n= 50-100. **p<0.01. Scale bars, 20mm



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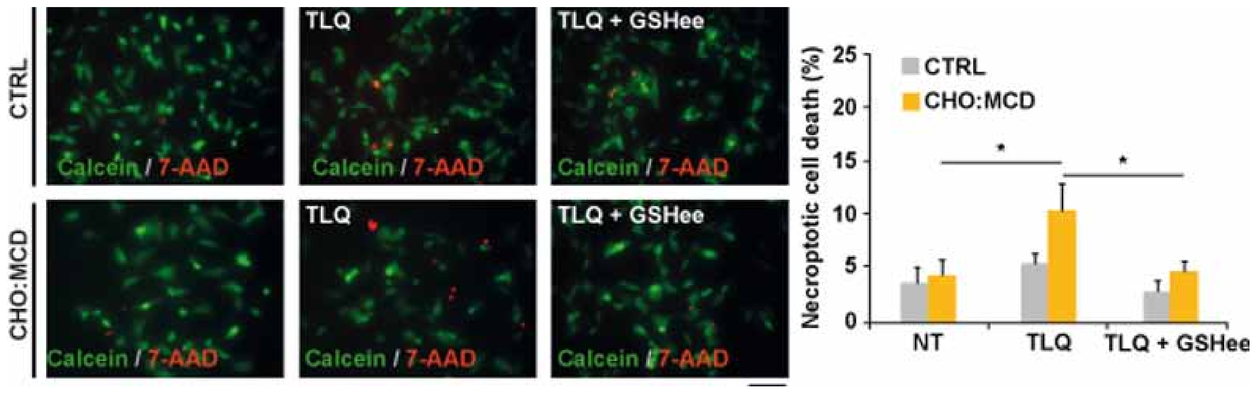
Mitochondrial GSH levels regulates necroptosis in cholesterol-enriched cells exposed to TNFα plus LCL-161 and qVD-OPh. GSH ethyl ester (GSHee) was incubated 30 min before the treatment with the necroptotic inducer. After treatment, cells were stained with Calcein AM and 7-aminoactinomycin D (7-AAD), which label lived and membrane-compromised cells, respectively. n= 50-100. *p<0.05. Scale bars, 20 mm.



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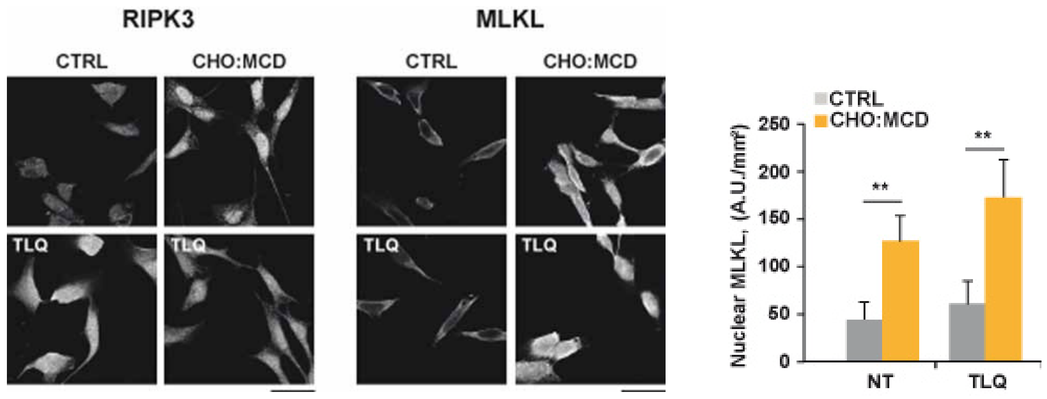
High intracellular cholesterol levels promotes nuclear localization of RIPK3 and MLKL. Representative confocal images of cells immunostained for RIPK3 and MLKL. Nuclei were counterstained with DRAQ5. Graphics show fluorescence intensity of MLKL that colocalizes with the nuclear stain. n= 10-20. Scale bars: 25 mm



CONCLUSIONS

High intracellular cholesterol levels compromise neuronal viability, promoting necrosome assembly and subsequent necroptosis, a pro-inflammatory type of cell death, which ultimately may contribute to chronic neuroinflammation in AD.

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