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LS-2-P-2121 Amyloid-β affected mitochondrial membrane as potential source for the autophagosome biogenesis

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Autophagy plays a crucial role in maintaining cellular homeostasis by carrying out continual degradation and recycling of cellular components [1]. However, overactive autophagy may promote neuronal cell death in neurodegenerative disorders including Alzheimer's disease (AD). Recent evidence has demonstrated that amyloid-β (Aβ) induces the formation of doubled-membrane-bound vacuole, known as the autophagosome [2]. In spite of various studies on the ultrastructure of autophagy, the membrane formation mechanism of autophagosome is still poorly understood. The purpose of this study is to identify the organelles that provide the source of membrane in the early autophagosome biogenesis. For this, we have performed immunocytochemical analysis, cryo-immunogold labeling and ultrastructural analysis, and three-dimensional electron tomography [3] in animal- and cell-model for AD. In the APP/PSEN1 transgenic (TG) mice that express human amyloid precursor protein, the deposition of Aβ plaques and distrophic neurite (DN) were detected in the hippocampus and cortex regions. We also identified the loss of peroxiredoxin, an endogenous cytoprotective antioxidant enzyme, and the accumulation of Aβ in the hippocampal mitochondria of transgenic mice with severe disruption of mitochondrial cristae. In the Aβ-stimulated murine microglial cell line, BV-2, we found that mitochondrial membranes were disrupted by the depletion of peroxiredoxin. In addition, the number of LC3, autophagosome marker, positive autophagosome was increased significantly within the cytoplasm. We also found that cytochrome c oxidase subunit IV (COX IV) and Beclin-1, a pre-autophagosome marker, were co-localized in tiny tubule-like structure and on some autophagosome membrane around the Aβ-affected mitochondria. These results suggest that the fragments of mitochondrial membrane are involved in the generation of pre-autophagosomal membranes during AD development. Our in vivo and in vitro studies strongly support the hypothesis that the mitochondria may be a potential source for the membrane biogenesis in the autophagosome formation.

References

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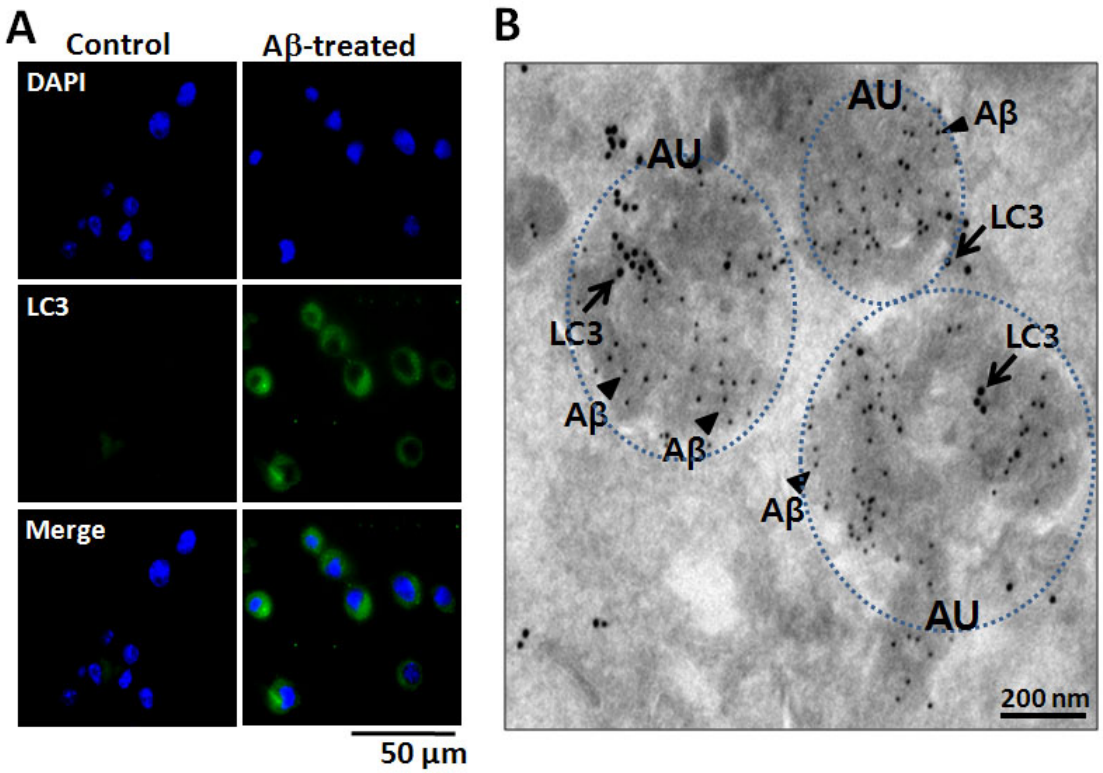

Fig. 1: Immunofluorescence and cryo-immunogold electron microscopy of LC3 in Ab-treated BV-2 cells. (A) LC3 positive signals were detected in cytoplasm and (B) LC3 were localized in autophagosome (AU) of Ab-treated BV-2 cells. Immunogold EM experiments were performed using anti-LC3 (15 nm) and anti-Aβ antibodies (10 nm).

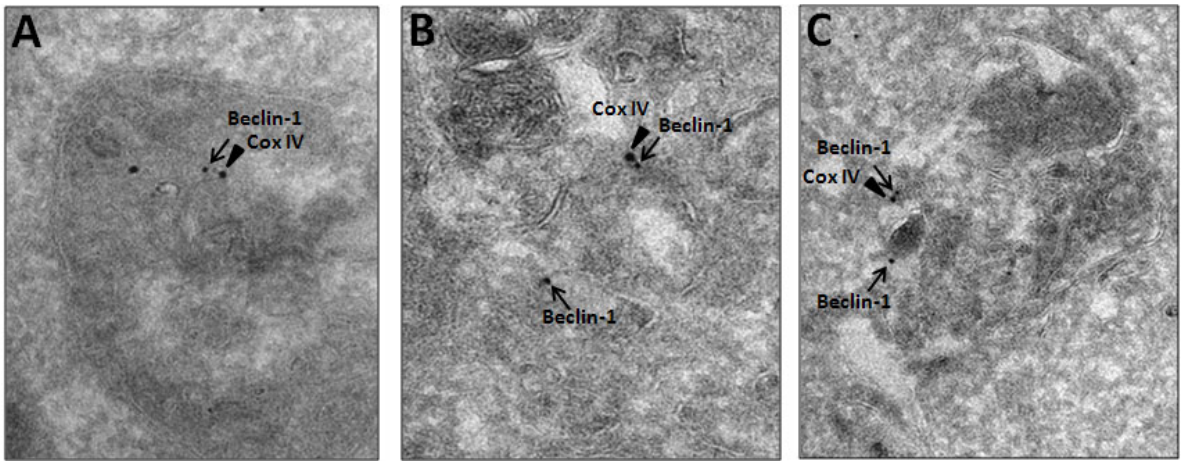

Fig. 2: Sequential double cryo-immunogold electron microscopy of Beclin-1 and CoxIV in Ab fibril-treated BV-2 Cells. In Ab-treated cells, both CoxIV (15 nm) and Beclin-1 (10 nm) were co-expressed in damaged mitochondria (A), fragmented mitochondria (B) and autophagosome membrane (C).