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Hydroxynonenal-Generated Crosslinking Fluorophore And Mitochondria-Derived Lipoic Acid Accumulation In Alzheimer Disease Reveal A Dichotomy Of Protein Turnover

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Light and electron immunohistochemical methods, when used in conjunction, allow for stringent analysis of the specific locations and relative levels of even non-protein based antigen detection. For instance, specific adducts of lipid peroxidation have been demonstrated to be associated with pathological lesions of Alzheimer disease (AD), suggesting oxidative stress is a major component in the disease [1]. Some HNE-protein products have physical and chemical properties similar to lipofuscin, linking lipid peroxidation and the lipofuscin accumulation that commonly occurs in neurons. Using light immunocytochemistry and immunoelectron microscopy, HNE-crosslinking modifications of the type that should accumulate in the lipofuscin pathway, were found in granulovacuolar degeneration (GVD) and Hirano bodies in AD (Figure 1A). These findings directly implicate lipid crosslinking peroxidation products as accumulating, not in the lesions or the lipofuscin pathways, but instead in a distinct pathway of GVD that accumulates cytosolic proteins. Mitochondrial abnormalities are also prominent features of AD [2]. The localization of lipoic acid, a sulfur-containing cofactor required for the activity of several mitochondrial enzyme complexes, was compared with cytochrome oxidase-1 in AD and control brain tissue. Using light microscopy, we observed that AD shows increased lipoic acid and cytochrome oxidase-1 immunoreactivity in the cytoplasm, and significantly, lipoic acid differed from cytochrome oxidase-1 in being associated with GVD-like structures (Figure 1B, inset). Ultrastructure analysis further demonstrated lipoic acid localized to mitochondria and cytosol, as well as autophagic vacuoles and lipofuscin in AD cases only (Figure 1B). In contrast, cytochrome oxidase-1 was limited to mitochondria and cytosol. These results indicate that mitochondria are key targets of increased autophagic degradation in AD [3]. Further, these findings highlight a possible dichotomy of protein turnover in AD, where membrane bounded-organelles are degraded in lysosome/lipofuscin and cytosolic proteins by an alternate pathway. When used together, light and electron microscopy continue to be significant tools for complete understanding of protein and non-protein antigenic targets, especially when studying abnormal cellular functions of disease [4].

References
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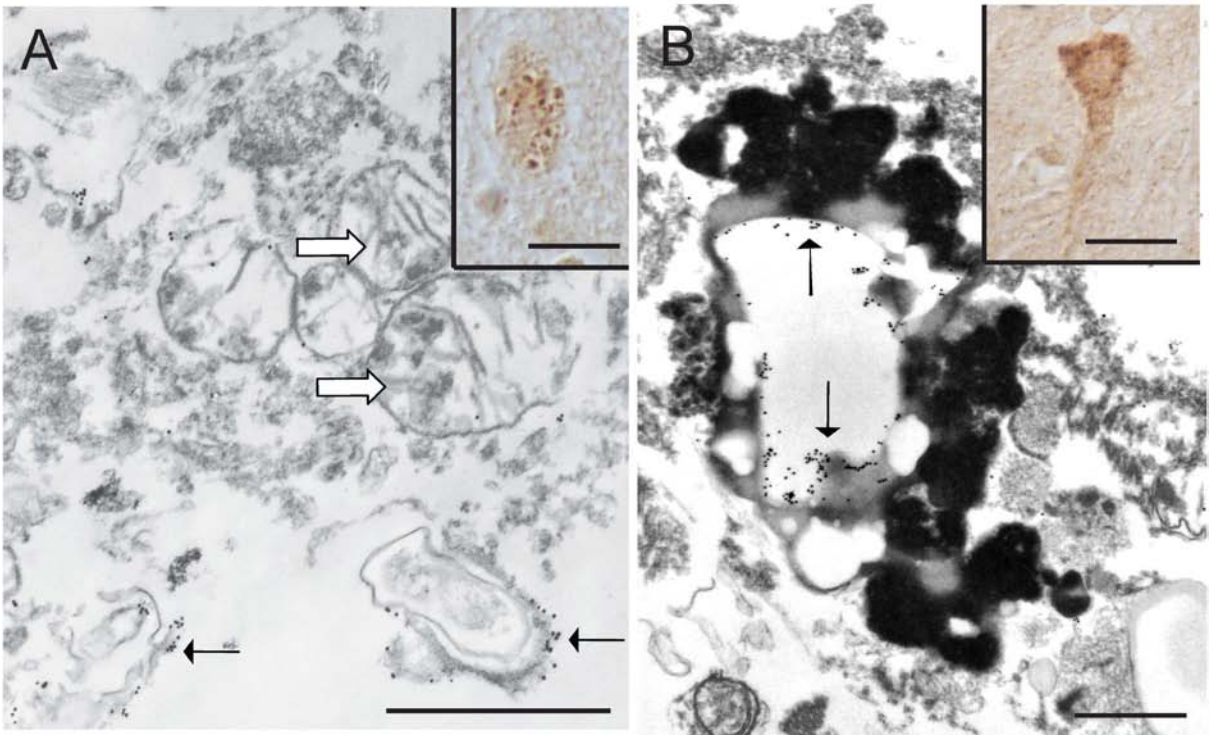

Figure 1. HNE-crosslinking modifications are distinctly localized to GVD in AD neurons (A, inset). Ultrastructurally, these epitopes, are found using gold-lableled immunoelectronmicroscopy, not in mitochondria (white arrows) of lipofuscin (not shown), but in GVD (arrows) consistent with light level findings (A). Lipoic acid is found in the neuronal cytoplasm and other granular structures in cases of AD (B, inset). Autophagic vacuoles associated with lipofuscin (B, arrows) are found to accumulate lipoic acid using gold-labeled immunoelectronmicroscopy. Scale bars: A,B = 1μm; insets = 20μm.

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