Morphological aspects of autophagy: Identification and possible mechanism of autophagosome formation in neuronal cells

Yasuo Uchiyama

Department of Cell Biology and Neuroscience, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan

y-uchi@juntendo.ac.jp
Keywords: autophagy, autophagosome, lysosome, cathepsins, Atg7

Autophagy, a cellular mechanism by which old and unneeded cellular constituents are segregated from the cytosol to form “autophagosomes” is induced in cells in response to various stresses (Figure 1). We have shown that deficiency in lysosomal proteinases such as cathepsin D or cathepsins B and L induces accumulation of lysosomes containing ceroid-lipofuscin; phenotypes of these mice resemble those of neuronal ceroid lipofuscinosis (NCL) [1-4]. In these mutant mice, the accumulation of abnormal lysosomal structures appears in accordance with an increase in the amount of membrane-bound LC3, a marker of “autophagosomes” in neurons. Such autophagosomes often contain granular osmiophilic deposits (GRODs), a hallmark of NCL, together with part of the cytoplasm, which contain undigested materials. These data strongly argue for a major involvement of autophagy in the pathogenesis of NCL. However, it remains largely unknown what signaling is essential for autophagosome formation. We have recently shown that massive loss of neurons is detected in the cerebral and cerebellar cortices of the mice lacking *Atg7*, an essential gene for autophagy in yeast, specifically in brains, while polyubiquitinated proteins accumulate in autophagy-deficient neurons as inclusions [5, 6]. This notion suggests that autophagy is involved in the degradation of ubiquitinated proteins. Moreover, p62/A170/SQSTM1 (p62), a multifunctional protein, is known to be capable of interacting with both ubiquitin and LC3, and is demonstrated to co-exist with ubiquitin in neurons deficient in Atg7 [7]. We therefore examined whether these three molecules are co-localized in cathepsin D-deficient neurons by immunofluorescent microscopy. Immunosignals for LC3 are co-localized in granular structures of cathepsin D-deficient neurons with those for ubiquitin and p62. Immunoelectron microscopy shows that membranes of GRODs are co-labeled with gold particles indicating ubiquitin and p62. These results suggest that ubiquitin labels GRODs with undigested materials and further binds p62. This ubiquitin/p62 assembly on the membrane of GRODs may happen to bind LC3 that is localized on the isolation membrane for autophagosomes, leading to the formation of autophagosomes. These autophagosomes further fuse with cathepsin D-deficient lysosomes and become GRODs via autolysosomes. Taken together, our data strongly indicate that this cycling of autophagosome formation contributes to the accumulation of lysosomes in neurons of mouse brains deficient in cathepsin D.

1. Koike M. et al., J. Neurosci. **20** (2000) p6898.
2. Nakanishi H. et al., J Neurosci. **21** (2001) p7526.
3. Koike M. et al., Mol. Cell. Neurosci. **14** (2003) p142.
4. Koike M. et al., Am. J. Pathol. **167** (2005) p1713.
5. Komatsu M. et al., J. Cell Biol. **169** (2005) p425.
6. Komatsu M. et al., Nature **441** (2006) p880.
7. Komatsu M, et al., Cell **131** (2007) p1149.

DOI: 10.3217/978-3-85125-062-6-371,

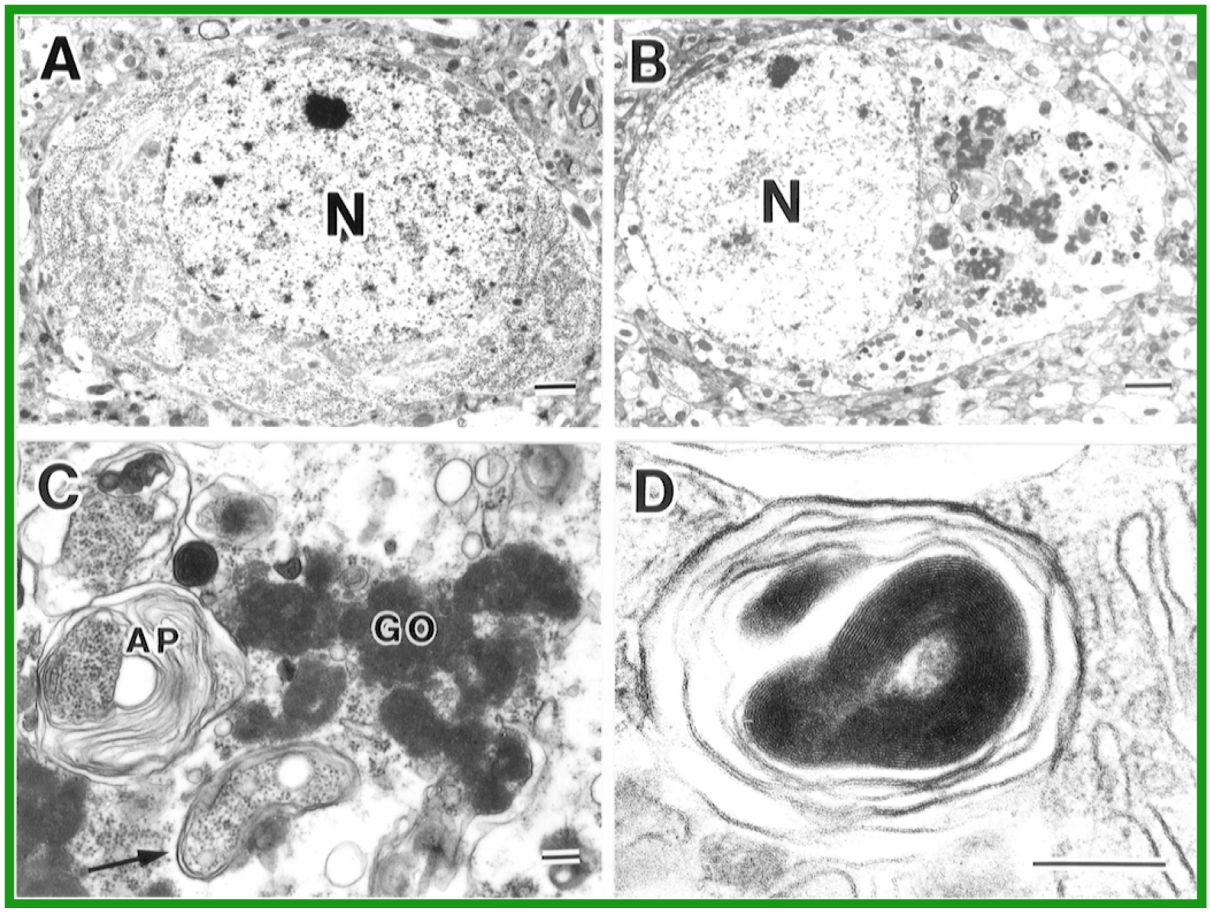

Figure 1. Electron micrographs of cerebral cortical neurons of mouse brains deficient in cathepsin D (B-D) and their control littermate (A) at 23 days after birth. B. Numerous dense granular structures/inclusion bodies are detected in the neuronal perikaryon. C. Granular osmiophilic deposits (GO) and single or double membrane structures that possess part of the cytoplasm with ribosomes are seen in the neuronal perikaryon. D. A membranous structure that show concentrically-arranged membranes resembling a finger print profile is demonstrated in a cortical neuron. Bars indicate 1 μm.

M.A. Pabst, G. Zellnig (Eds.): MC2009, Vol. 2: Life Sciences,
DOI: 10.3217/978-3-85125-062-6-371, © Verlag der TU Graz 2009