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Biochemical and Biophysical Research Communications 339 (2006) 485–489
BBRC
www.elsevier.com/locate/ybbrc

Organelle degradation during the lens and erythroid differentiation is independent of autophagy

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Received 28 October 2005
Available online 15 November 2005

Abstract

Autophagy is a bulk degradation system within cells through which cytoplasmic components are degraded within lysosomes. Primary roles of autophagy are starvation adaptation and intracellular protein quality control. In contrast to the ubiquitin–proteasome system, autophagy can also degrade organelles. Here we examined a possible role of autophagy in organelle degradation during lens and erythroid differentiation. We observed that autophagy occurs in embryonic lens cells. However, organelle degradation in lens and erythroid cells occurred normally in autophagy-deficient *Atg5*$^{-/-}$ mice. Our data suggest that degradation system(s) other than autophagy play major roles in organelle degradation during these processes.

Keywords: Autophagy; Lens; Erythroid cell; Organelle degradation; Lysosome

Autophagy is a generic term for the degradation of cytoplasmic content by lysosomes [1–4]. There are three types of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy. The most extensive molecular dissection and physiological studies have been performed for macroautophagy. Simply referred to as autophagy, macroautophagy is ubiquitously observed in eukaryotes. This process is mediated by a unique organelle termed the autophagosome. Upon the induction of autophagy, membrane cisternae (called isolation membranes or phagophores) enclose a portion of the cytoplasm, resulting in the formation of the double membrane-bound autophagosomes [5]. The autophagosome subsequently fuses with lysosomes to become an autolysosome (autophagolysosome); within this structure, the cytoplasm-derived contents are degraded by lysosomal hydrolytic enzymes.

Autophagy is upregulated in mice following food withdrawal [6], and in neonates, who face physiological starvation due to the sudden termination of transplacental nutrients [7]. We generated mice deficient for Atg5, a protein known to be essential for autophagosome formation [8,9]. *Atg5*$^{-/-}$ mice appear normal at birth, but exhibit systemic amino acid and energy insufficiency, and die within 10 h of birth [7]. Thus, the induction of autophagy during the early neonatal starvation period is an important physiological response. Recently, *Atg7*$^{-/-}$ mice were reported to exhibit a similar phenotype [10]. In addition to the function of induced autophagy in starvation adaptation, basal autophagy seems to have a critical role in intracellular protein quality control under normal conditions; liver-specific *Atg7*$^{-/-}$ mice showed accumulation of cytoplasmic inclusion bodies that are positive for ubiquitin [10].

In contrast to the ubiquitin–proteasome system, autophagy can degrade not only soluble proteins but also intracellular organelles such as mitochondria [11,12], peroxisomes

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0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.11.044

[13,14], and endoplasmic reticulum [15,16]. Some of these organelles are recognized selectively [13,14]. Moreover, abnormal organelles were observed in autophagy-deficient hepatocytes, suggesting that autophagy might be involved in quality control of intracellular organelles [10].

In addition to the normal turnover of intracellular organelles, dramatic degradation of organelles is observed in the processes of lens and erythroid development. The lens contains two types of cells, the epithelial cells covering the anterior surface of the lens and the fiber cells that differentiate from the epithelial cells. During late embryogenesis, membrane-bound organelles within the epithelial cells are rapidly lost, which allows fiber cells to be transparent [17–19]. Similarly, intracellular organelles are eliminated during erythroid cell maturation. The mechanism by which these organelles are destroyed during this process is not fully understood. The presence of autophagic vacuoles in these cells suggested the involvement of autophagy in the organelle loss [20–24]. Furthermore, it was recently reported that the degradation of nuclei of lens cells depends on DNase II-like acid DNase/DNase IIβ [25], suggesting that chromatin degradation occurs in an acidic organelle, possibly in the lysosome. Therefore, we addressed this issue using autophagy-indicator mice [6] and autophagy-deficient mice [7].

Materials and methods

Mice. *Atg5*$^{+/-}$ and *Atg5*$^{-/-}$ mice on the C57BL/6 background have been described previously [7]. *Atg5*$^{+/-}$ mice were crossed with green fluorescent protein (GFP)-fused LC3 transgenic mice [6] to produce *Atg5*$^{-/-}$ mice expressing GFP-LC3 (GFP-LC3 *Atg5*$^{-/-}$). For caesarian delivery, pregnant mothers were injected on 17.5 and 18.5 dpc with 2 mg progesterone (Luteum Injection, Teikoku Hormone, Tokyo) to delay birth; neonates were obtained at 19.5 dpc. To prolong the survival of *Atg5*$^{-/-}$ neonates, artificial milk feeding was performed every 3–6 h as previously described [7]. All animal experiments were approved by the Institutional Committee of the Tokyo Metropolitan Institute of Medical Science.

Immunohistochemical analysis. Embryos and neonates were examined by immunohistochemical analysis. Mice were perfused transcardially with 4% PFA in PB (pH 7.4). Tissues were postfixed overnight in the same fixative and embedded in either Tissue-Tek O.C.T. compound (Sakura Finetechnical Co., Ltd.) or paraffin. GFP-LC3 localization was analyzed as previously described [6]. For immunofluorescence microscopy, after blocking in either 5% bovine serum albumin (BSA) in PBS, lens sections were incubated with a mouse anti-KDEL monoclonal antibody (Stressgen) for 1 h, followed by a 30 min incubation with AlexaFluor 488-conjugated goat anti-mouse IgG (H + L) antibody (Molecular Probes). Sections were analyzed using microscopes (Olympus IX81) equipped with CCD cameras (ORCA ER, Hamamatsu Photonics).

Electron microscopy. Lens and circulating erythrocyte pellets were fixed with 2.5% glutaraldehyde in 0.1 M PB (pH 7.4) for 2 h. Conventional electron microscopy was performed as previously described [26].

Results and discussion

Since the organelle free zone (OFZ) in the lens is created between 17.5 dpc and birth, we first determined the occurrence of autophagy at this stage. LC3, a mammalian homolog of yeast Atg8, localizes to autophagosome membranes as a phosphatidylethanolamine (PE)-conjugated form [27,28]. Taking this advantage, we have developed an autophagy-indicator mouse model, in which GFP-LC3 is systemically expressed [6]. Using these mice, autophagosomes can be detected as GFP-positive dots [29]. In lens of 17.5 dpc embryos, a number of GFP-LC3 dots were observed at the center region (Figs. 1A and B). Fewer

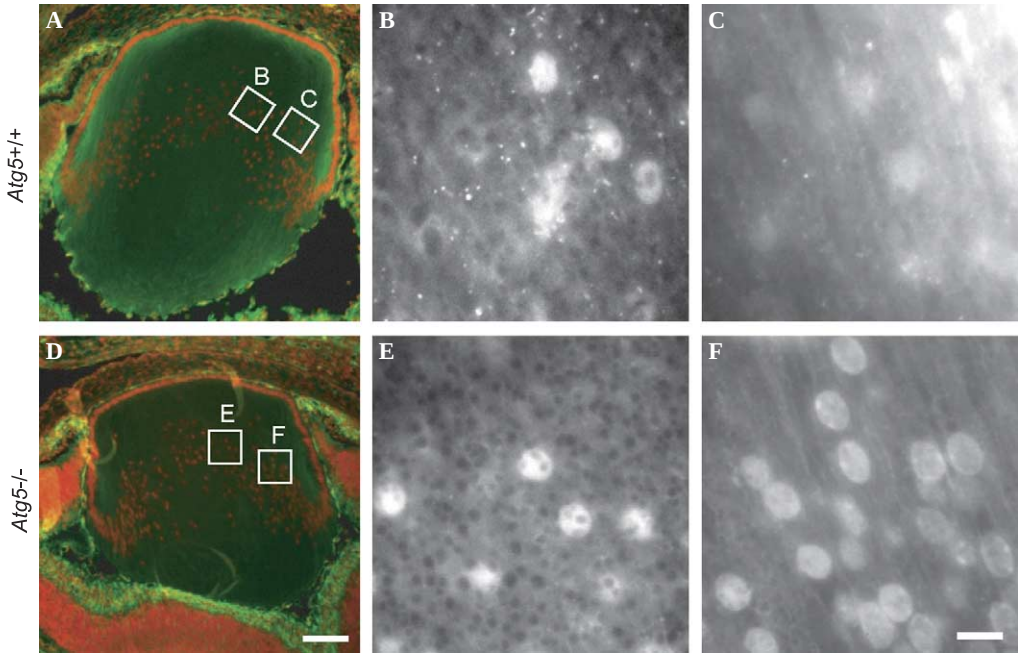

Fig. 1. GFP-LC3 localization in embryonic lens. Lens were isolated from 17.5-dpc embryos of heterozygous GFP-LC3 transgenic mice on the *Atg5*$^{+/+}$ (A–C) or *Atg5*$^{-/-}$ (D–F) backgrounds. Lens were immediately fixed and sectioned. Nuclei were stained with Hoechst 33258 (shown in red in A,D) and analyzed by fluorescence microscopy. (B,C,E,F) Higher magnification images of the GFP signals in the indicated regions in (A,D). Scale bar, 100 μm (A,D) and 10 μm (B,C,E,F).