Impaired G1-Arrest, Autophagy, and Apoptosis in *Atg7*-Knockout Mice

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Atg7 Modulates p53 Activity to Regulate Cell Cycle and Survival During Metabolic Stress

Lee et al

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Exit from cell division cycle, induction of autophagy, and activation of apoptosis in response to metabolic stress occur simultaneously or sequentially. However, the molecular interrelation(s) between these intracellular events remains obscure. Recent work by Finkel et al[1] provides evidence that Atg7, an autophagy-related protein, regulates G1-arrest by interacting with p53 and that p53-mediated apoptosis is activated under autophagy-deficient conditions.

Macroautophagy (hereafter referred to as autophagy) is a self-eating system conserved among eukaryotes. In this process, cellular components, including organelles, are entrapped into a double-membrane structure called the autophagosome and then degraded by lysosomal hydrolases (Figure 1).[2] In addition to its role in supplying amino acids in response to nutrient starvation, autophagy is involved in quality control to maintain cell health. Thus, inactivation of autophagy results in the accumulation of cytoplasmic protein aggregates of misfolded proteins and damaged and degenerated organelles, which compromise cell function and often result in life-threatening diseases.[2]

The molecular underpinnings of autophagosome formation were uncovered mainly in yeast *Saccharomyces cerevisiae*. To date, genetic studies of *S. cerevisiae* have identified >30 autophagy-related (*ATG*) genes, 18 of which are core *ATG* genes that are essential for autophagosome formation.[3] Importantly, core *ATG* genes are well conserved in mammals, where the functions of the encoded proteins markedly overlap with the corresponding proteins in yeast, although a few mammal-specific proteins have been identified. Atg proteins are categorized into several subclasses, but they function sequentially and cooperatively to regulate autophagosome formation (Figure 1).[4] The ubiquitin-like modifiers Atg12 and LC3 (mammalian Atg8 homolog) are activated by the E1-like enzyme Atg7 and transferred to 2 different E2-like enzymes: Atg10 and Atg3, respectively. Whereas Atg12 forms an isopeptide bond with Atg5, LC3 forms an amide bond with phosphatidylethanolamine (PE) in a reaction dependent on the Atg12-Atg5 conjugation. Atg16L forms a high-molecular-weight complex with Atg12-Atg5. The Atg12-Atg5-Atg16L complex functions as an E3-like enzyme, determining the site of LC3 lipidation.[4] Although the Atg12-Atg5-Atg16L complex is required for elongation of the isolation membrane, the phosphatidylethanolamine-bound LC3 (LC3-II) is thought to be important for membrane biogenesis and closure of the isolation membrane (Figure 1).[4]

In a recent article published in *Science*, Lee et al[1] reported that in primary mouse embryonic fibroblasts, loss of *Atg7* abrogated G1-arrest in response to metabolic stress and contact inhibition. Surprisingly, depending on the nature of metabolic stress, Atg7 interacted with p53 directly, and this complex then bound to the promoter region of *Cdkn1a*, a cyclin-dependent kinase inhibitor (Figure 2). These events resulted in modulation of *Cdkn1a* expression and inhibition of entry into the S-phase. Paradoxically, the same group noticed the upregulation of p53-dependent proapoptotic genes, such as *Puma*, *Bax*, and *Noxa*, in primary *Atg7*$^{-/-}$ mouse embryonic fibroblasts. Autophagy-deficient mouse embryonic fibroblasts showed increased production of reactive oxygen species and DNA damage, which activated the DNA damage sensor, ataxia teleangiecstasia mutated kinase. Subsequently, the mediator molecule *Chk2* phosphorylated Ser20 of p53, followed by induction of a series of proapoptotic genes (Figure 2). As expected, changes in the expression of these genes were completely blocked by the additional loss of *Chk2*. The phosphorylation of p53 and enhanced cell death observed in *Atg7*$^{-/-}$ mouse embryonic fibroblasts were restored by simultaneous depletion of *Chk2*. Furthermore, the neonatal lethality of *Atg7*-knockout mice was rescued, at least in part, in *Atg7 Chk2*-double knockout mice.

Finkel et al[1] have suggested that the interaction of Atg7 with p53 in response to metabolic stress and contact inhibition is a rate-limiting step for the induction of *Cdkn1a*, followed by G1-arrest (Figure 2). What are the factors that enhance the Atg7-p53 interaction? Evidence suggests that acetylation plays an important role in autophagy.[5,6] Previous studies indicated that Sirt1 deacetylates several Atg proteins, including Atg7, which is indispensable for starvation-induced autophagy,[7] and conversely that p300 acetyltransferase directly interacts with and acetylates Atg7 to inhibit autophagy.[8] Beyond autophagic regulation, deacetylation of Atg7 might promote the interaction with p53 to induce *Cdkn1a* gene expression. Because the structures of both Atg7 and tetramerization domain of p53 have been analyzed,[9,10] determination of the structure of the

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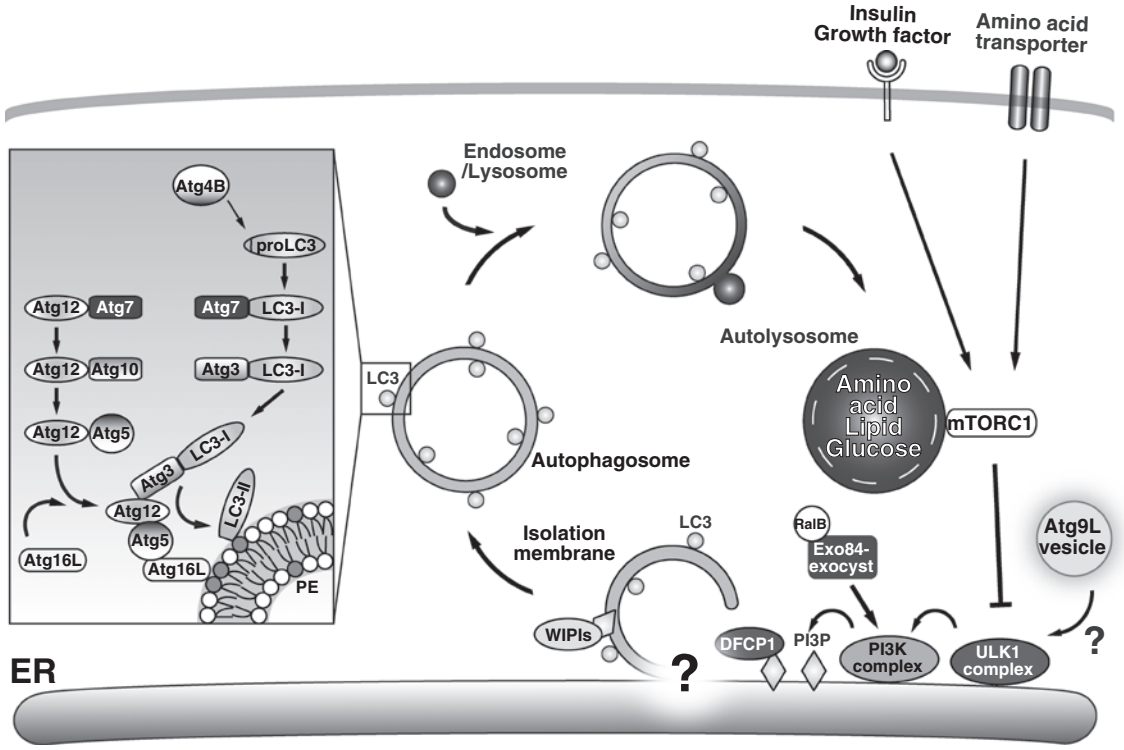

Figure 1. Autophagy. The initial steps of autophagy include the formation and subsequent elongation of the isolation membrane. The isolation membrane then enwraps various cytoplasmic constituents, such as organelles, until its edges fuse with each other to form a double-membrane structure called the autophagosome. Finally, the outer membrane of the autophagosome fuses with the lysosome and endosome. The sequestered cytoplasmic components, together with the inner membrane of the autophagosome, are completely degraded by lysosomal hydrolases (Mizushima et al[4] for details on the molecular players). The ubiquitin-like modifier, LC3, covalently conjugates with phosphatidylethanolamine (PE) through an enzymatic cascade consisting of Atg7 (E1-like enzyme), Atg3 (E2-like enzyme), and Atg12-Atg5-Atg16L complex (function as an E3-like enzyme). The PE-conjugated LC3, named LC3-II, is localized to the inner and outer membranes of the isolation membrane and is essential for membrane biogenesis and closure of the isolation membrane. The activated ULK1 complex regulates class III PI3K complex and the multimembrane spanning protein Atg9L at pre-autophagosomal structure/phagophore-assembly site (PAS) close to the endoplasmic reticulum (ER), which is involved in the formation of the omegasome. PI3P-binding WIPIs, Atg12–Atg5–Atg16L1 complex, and LC3–PE conjugate play important roles in the elongation and closure of the isolation membrane/phagophore. ER indicates endoplasmic reticulum; PI3P, phosphatidylinositol 3-phosphate.

Atg7-TET domain complex is feasible. Structural analysis should enhance our understanding of the role of Atg7 in cell cycle regulation.

What is the physiological importance of the p53-Atg7 pathway in the regulation of cell cycle arrest? Exit from cell division cycle (ie, upregulation of *Cdkn1a*) seems to be dependent on Atg7 but not other Atg proteins, such as Atg5 and Beclin 1, indicating an additive function of Atg7 beyond autophagy. Hence, loss of *Atg7* in mice should be associated with worse phenotypes than other *Atg*-knockout mice. Nevertheless, to date, we have not observed any profound phenotypes of *Atg7*-knockout mice compared with those of *Atg5*- or *Atg3*-knockout mice.[11–13] These mutant mice are born in accordance with Mendelian inheritance ratios and survive but have lower amino acid levels in their sera and tissues and die within the first day of life. Further in vivo add-back experiment of mutant Atg7, which is defective in interaction with p53, but capable of induction of autophagy, will be critical for understanding the physiological significance of the p53-Atg7 pathway.

Overcoming neonatal death in autophagy-deficient mice by additional loss of *Chk2* is surprising because the autophagy-deficient mice have multiple systemic defects, including neurological abnormalities and impaired trophic dynamics.[11,14] Finkel et al[1] have shown the induction of proapoptotic pathways only in livers of *Atg7*$^{-/-}$ neonate mice. The livers of neonate mice contain a large number of hematopoietic stem cells and related cells, such as erythroid cells. Recent studies have shed light on the importance of autophagy in mitochondrial homeostasis (mitophagy)[15] and indicated that defective mitophagy in hematopoietic cells is directly linked to rapid cell death. In fact, impaired autophagy in hematopoietic stem cells, T cells, and B cells causes acute cell death because of the accumulation of damaged mitochondria, followed by production of reactive oxygen species.[16,17] Therefore, suppression of cell death in these cells by additional loss of *Chk2* is plausible. What about other tissues? The authors asserted that there was neurodegeneration in *Atg7 Chk2*-double knockout mice. However, partial rescue of neurological defect in autophagy-deficient mice is expected because loss of *Chk2* restores the suckling disorder in *Atg7*$^{-/-}$ mice (the double-mutant mouse cannot survive >1 day unless it overcomes this defect).

Imbalance between cell growth and death contributes to tumorigenesis. Mice heterozygous for *Beclin 1* mutation, systemically mosaic for *Atg5* deficiency, or lacking *Atg7* specifically in liver tissue develop liver tumors, probably because of dysregulation of signal transduction pathways, as

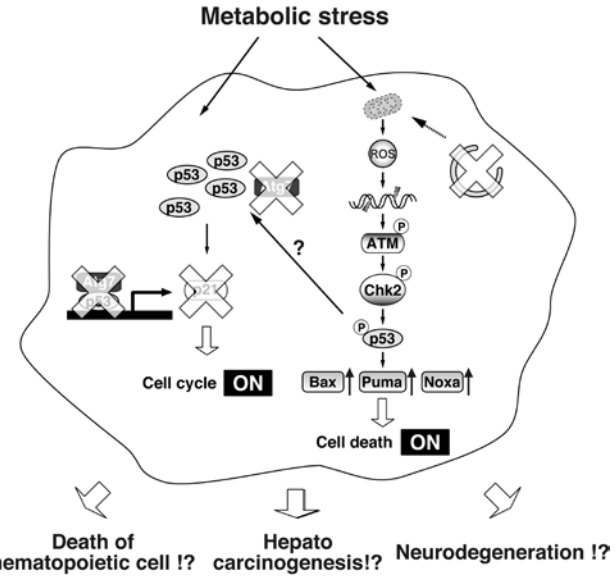

Figure 2. Schematic model of cell cycle and cell death in *Atg7*-deficient mouse embryonic fibroblasts (MEFs) under metabolic stress conditions. In response to metabolic stress, the p53-Atg7 complex binds to the promoter region of *Cdkn1a* to induce its expression, leading to G1-arrest. Lack of *Atg7* abrogates the induction of *Cdkn1a* and exit from cell division cycle. Meanwhile, accumulation of DNA damage through impaired mitochondria homeostasis in *Atg7*-knockout mice activates DNA damage sensor, ATM kinase. Subsequently, Chk2 phosphorylates Ser20 of p53, followed by induction of a series of proapoptotic genes. Imbalance between cell division and cell death could lead to hematopoietic cell death, neurodegeneration, and tumorigenesis. ROS indicates reactive oxygen species.

well as impaired organelle quality control.[18–21] The growth of liver adenoma in mice with liver-specific knockouts of *Atg7* is markedly suppressed when *p62* is also knocked out, because marked accumulation of p62, due to loss of autophagy, leads to dysregulation of nuclear factor-κB signaling, activation of apoptosis, and responses to environmental stress.[22,23] However, loss of *p62* does not suppress hepatocarcinogenesis itself. The autophagy-related p53 pathways might be engaged in tumorigenesis (Figure 2). Further studies are needed to determine the state of tumorigenesis in *Atg7 Chk2*-double deficient mice.

The report by Finkel et al[1] helps our understanding of the molecular mechanism by which impaired autophagy and Atg-related proteins are involved in apoptosis and cell cycle arrest. However, pathophysiological significance of the regulation remains unclear as described in this commentary. With the recent development of sophisticated research tools, such as Cre-mediated conditional knockout techniques, it has become clear that loss of autophagy is associated with various life-threatening diseases, such as neurodegeneration, cardiomyopathy, liver injury, glomerulosclerosis, myopathy, and cancer.[2] Further studies are needed to clarify the pathological role of proapoptotic genes in autophagy-deficient tissues in these diseases (Figure 2).

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Disclosures

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