Subcellular Processes in Plants and Animal Cells

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Discovery and functional analysis of the novel regulators of autophagosome formation and maturation

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Autophagy is a biological process allowing the cell to recycle long-lived proteins and damaged organelles. Following sequestration in double or multimembrane autophagic vesicles, the cargo is delivered to lysosomes for degradation. This phenomenon ensures cell survival under stress conditions and plays an important role in the elimination of abnormal or misfolded proteins and damaged organelles [1]. Autophagic vesicle formation and maturation relies on around 30 ATG proteins. Yet, recent studies indicate the presence of an complex network integrating the basic autophagy pathway into fundemental cellular events allowing a coordinated response in times of stress [1, 2].

In our lab in Sabanci University, we focus on signaling events regulating mammalian autophagy and connecting it to cellular pathways. We discovered a role for the Death-Associated Protein Kinase in apoptosis and autophagy crosstalk during drug-induced endoplasmic reticulum stress (Figure 1) [3]. To discover new autophagy regulators and coordinators, we performed several unbiased functional screens. Our search for caspase cleavage sites in autophagy proteins revealed a caspase-8-mediated autophagy regulation mechanisms during death receptor activated cell death [4]. Our microRNA screens led to the discovery of several miRNAs targeting autophagy at various steps of the pathway (Figure 2) [5, 6]. miRNAs are able to affect the expression of a number of proteins at once. Therefore, miRNA networks seem to integrate cellular stress response pathways including autophagy and coordinate them to shape cell faith. We also discovered novel proteins involved in autophagy regulation. In fact, some of these proteins were directly interacting with core autophagy machinery components [7]. Unexpected direct links between autophagy and other cellular pathways were found, allowing us to reveal new entry points for autophagy regulation and coordination in cells. Results from these studies including those obtained using fluorescent confocal microscopy and transmission electron microscopy will be presented.

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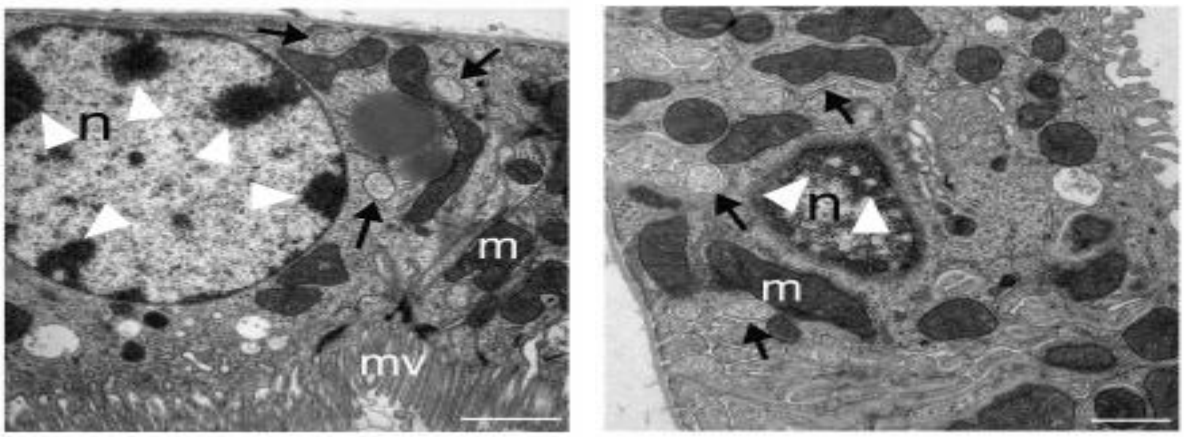

Figure 1. ER stress induces hallmarks of apoptosis and autophagy in vivo in the intact kidney. Ultrastructural analysis of ER stress-induced autophagy and apoptosis features in the kidney by TEM. Shown are images of kidneys from tunicamycin injected mice. Scale bar, left panel, 2µm; right panel, 1µm. n, nucleus, mv, microvilli. Arrows, double membrane bounded autophagic vesicles. Note extensive chromatin condensation in the nuclei (arrowheads) and mitochondrial condensation (m) indicative of apoptotic pathway activation in cells with autophagic vesicles.

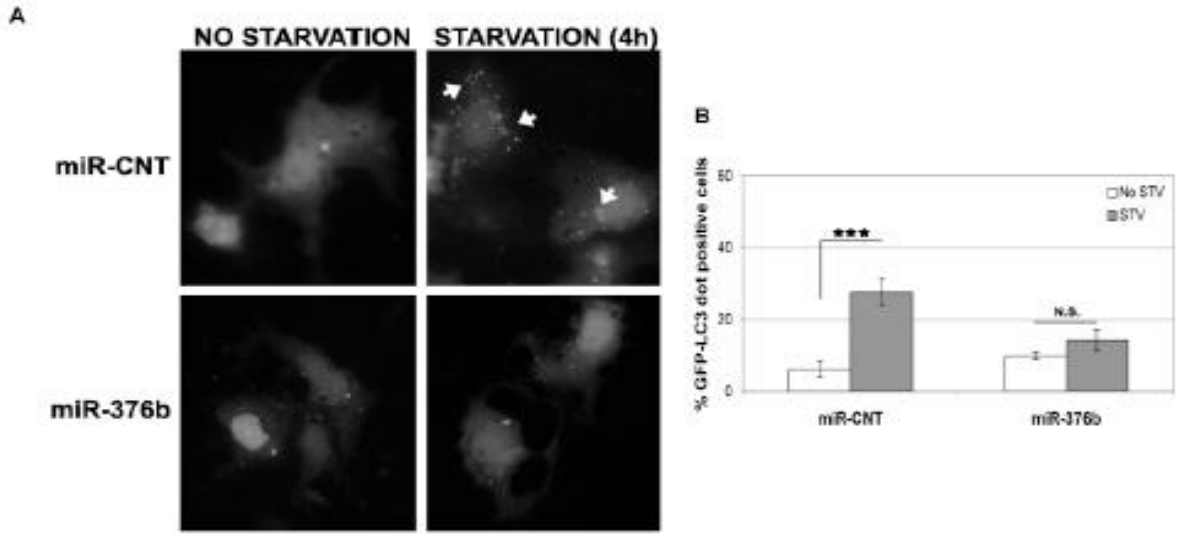

Figure 2. Blockage of starvation-induced autophagy in Huh-7 cells shown using fluorescent confocal microscopy. (A) miR-376b blocked starvation-induced autophagy (GFP-LC3 dot formation). White arrows indicate clusters of the GFP-LC3 dots in cells. (B) Quantitative analysis of GFP-LC3 dot positivity (mean ± S.D. of independent experiments, n=3, ***p<0,01. N.S., not significant.).