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Autophagy regulation by miRNAs: when cleaning goes out of service

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Autophagy is a degradation pathway, recycling cell components in a number of basal and sublethal stress conditions. The formation of specific vesicles (autophagosomes), carrying their content to the lysosome, is primed by several post-translational molecular events, including protein modification and translocation to the ER or other autophagy start sites. However, less is known about autophagy transcriptional and post-transcriptional regulation. In particular, the microRNA (miRNA) world, which plays crucial roles in development, signalling and stress response in eukaryotes, has not yet been directly linked to autophagy. A paper in this issue of *The EMBO Journal* reveals an *miR-101*-specific downregulation of *STMN1*, *RAB5A* and *ATG4D* genes, leading to an efficient decrease of autophagy. By this inhibition, *miR-101* sensitizes resistant breast cancer cells to death. Thus, this work provides an exceptional insight into different novel levels of autophagy regulation and emphasizes the role that miRNAs can play in biomedicine.

Intracellular clearance is performed by eukaryotic cells in basal conditions and upon a plethora of stress stimuli, ranging from nutrient starvation, to pathogenic invasion or to mechanical, oxidative and metabolic stress (Kroemer *et al*, 2010). Besides the digestion of individual ubiquitylated proteins, mediated by the proteasome machinery, autophagy is the main route to degradation and recycling for damaged organelles and long-lived proteins and its role has been demonstrated in a number of pathological conditions. Indeed, in cancer, autophagy may play a dual, and apparently contradictory, role (Mathew *et al*, 2007): (a) mutations in autophagy genes may disable cells from a proper clearance of damaged organelles, inducing ROS accumulation and DNA damage, followed by chromosomal instability; (b) in cancer cells, which originated by a different set of mutations *not* involving autophagy, this process may represent a chance of survival to any kinds of anti-cancer stressors. Currently, >35 proteins are believed to be essential for autophagy occurrence and progression (Kroemer *et al*, 2010). They regulate autophagosome priming, that is, the formation of the vesicles that engulf targets for degradation, and their elongation and fusion to the lysosome where recycling takes place. In particular, the mTOR-dependent serine–threonine kinase ULK1 is part of a multimolecular complex, drives phosphorylation and promotes translocation of a number of pro-autophagic molecules. An autophagy *core* complex, formed by BECLIN 1, its kinase VPS34 and their cofactor AMBRA1, promotes the membrane modification required for autophagosome assembly, while a ubiquitin-like conjugation system based on ATG5, ATG7 and ATG12 mediates autophagosome completion. A number of these proteins are regulated at a post-translational level; they are called into action by ULK1, AMPK, alternative kinases (such as ERK2), or by Sirtuin-mediated acetylation.

However, in the gene-to-protein direction, several gaps in the autophagy regulative pathways are present. At a transcriptional level, recent data point to a crucial role for the transcription factor TFEB in the synergic activation of the cell-clearance network, including autophagy and lysosomal genes (Settembre *et al*, 2011). More specific transcription factors, however, can activate specific key autophagy genes in response to a variety of stimuli. As a recent example, in the nematode *Caenorhabditis elegans*, the transcription factor Atf-2 is able to directly upregulate the expression of at least two key genes related to autophagy, *bec-1/ATG6* and *lgg-1/ATG8* (Erdélyi *et al*, 2011).

On the way down to protein synthesis, miRNAs represent an important and still relatively unexplored world of regulation. miRNAs do not encode for proteins, but exert catalytic, structural or regulatory activities by annealing to specific target RNAs, and by downregulating their stability and/or translation (Inui *et al*, 2010). Besides their functions in development and differentiation, >150 miRNAs were reported in recent years as being either upregulated or downregulated in cancer, while miRNA expression profiling of human tumours has identified hallmarks associated with diagnosis, staging, progression, prognosis and response to chemotherapy.

One of the first demonstrations of the role played by miRNAs in autophagy regulation is provided in this issue of *The EMBO Journal*, by Frankel *et al* (2011). By means of a functional screen in breast cancer cells, aimed at identifying miRNAs regulating the autophagy flux, the authors identified the miRNA *miR-101* as a strong inhibitor of basal and induced autophagy, able to put the cell-clearance network out of service (see Figure 1). They went on with the analysis using a transcriptome profiling assay and found at least three *bona fide* targets of *miR-101*: Stathmin 1 (STMN1), the RAB GTPase 5A (RAB5A) and the autophagy-related protein

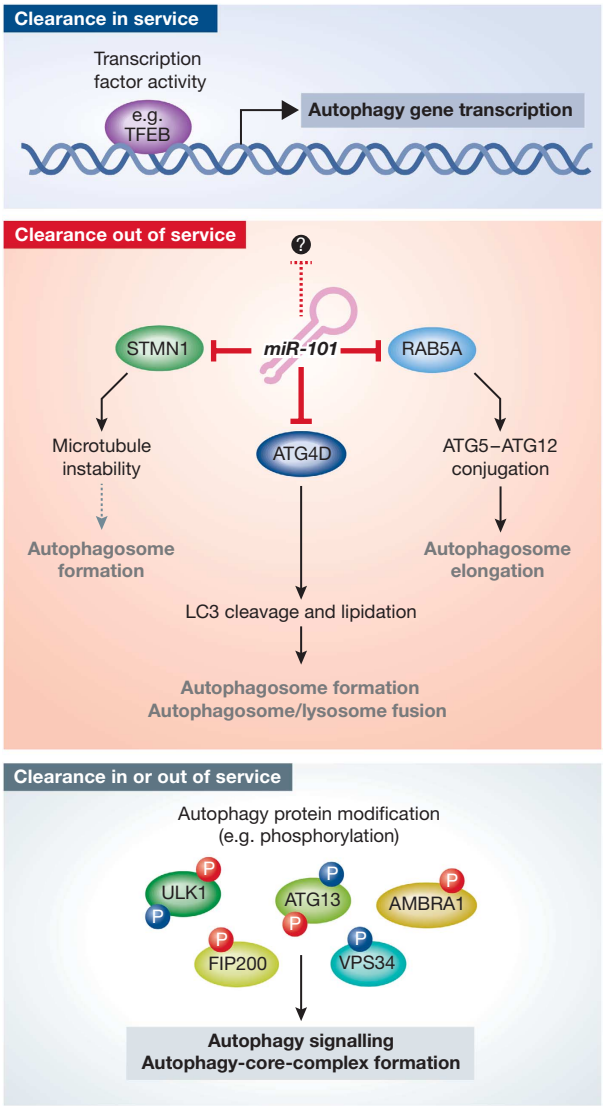

Figure 1 Another level is added to the regulation of autophagy-mediated intracellular clearance: *miR-101* inhibits STMN1, ATG4D and RAB5A, triggering different steps of an autophagosome's life. The dashed/grey lines point to unclear modes of action or unknown factors. Transcriptional regulation (pro-autophagic) is exemplified in the upper panel by the pan-autophagic factor TFEB, while the phosphorylation of a number of pro-autophagic proteins is illustrated in the lower panel, to summarize post-translational regulation (the autophagy–core complex proteins AMBRA1 and VPS34; the ULK1 complex factors ATG13 and FIP200). VPS34, ATG13 and ULK1 are regulated both positively and negatively by phosphorylation (blue and red phosphate, P).

4D (ATG4D). These genes were then downregulated by siRNA to demonstrate their link to the autophagy process. This confirmation phase was, indeed, crucial: In contrast to ATG4D, an enzyme relatively important in the cleavage and lipidation of the autophagy structural and functional factor LC3B both at the onset and termination of the autophagosome *itinerary* (Kaminskyy and Zhivotovsky, 2011), both RAB5A and STMN1 had previously uncertain roles in autophagy. RAB5A was shown to regulate ATG5–ATG12 conjugation in the autophagosome completion pathway (Ravikumar *et al*, 2008), while STMN1 destabilizes microtubules and plays an important role in cell-cycle regulation (Cassimeris, 2002). As for the latter, it should be noted that the autophagy core complex is normally sequestered to the microtubules, where it is bound through its component AMBRA1 to the dynein light chain DLC1, and from where it can be unleashed by ULK1-dependent AMBRA1 phosphorylation (Di Bartolomeo *et al*, 2010). STMN1 upregulation, observed in a high variety of cancers, could depend on *miR-101* loss, somehow disabling this step of autophagy regulation. Notably, prompted by the novelty of STMN1 in the complex scenario of autophagy, the authors used a complementation assay, to confirm the importance of this target in the *miR-101*-dependent regulation.

This highly interesting paper leads to another important consideration: autophagy can be potently inhibited by *miR-101*, sensitizing cancer cells to cell death. Frankel *et al* (2011) used 4-hydroxytamoxifen (4-OHT) to induce cell death in breast-cancer-derived MCF-7 cells (a stimulus to whom they are usually resistant), synergistically with *miR-101* as an autophagy inhibitor. Most likely, at least in breast cancer cells, elevated levels of autophagy, due to the progressive loss of *miR-101*, have the potential to trigger cancer cell survival. Indeed, cells devoid of *miR-101* could cope with metabolic stress and eventually regrow after any anti-cancer treatments. Interestingly, besides the tumour-suppressive roles of *miR-101* in several cancers, *ATG4D*, *RAB5A* and *STMN1* are all described as genes with oncogenic potentials.

In conclusion, the study heralds novel intriguing levels of regulation for the autophagy process. Pro-autophagic mRNAs could be downregulated by other miRNAs besides *miR-101*, and more autophagy regulators could be discovered as targets, by diving into the deep water of the RNA world. Furthermore, alternative therapeutic opportunities can pop out by the systematic analysis of this cancer-related class of molecules in the context of autophagy regulation.

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Conflict of interest

The author declares that he has no conflict of interest.

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