Mitochondrion 19 (2014) 58–68

Contents lists available at ScienceDirect

Mitochondrion

journal homepage: www.elsevier.com/locate/mito

Autophagy and mitophagy interplay in melanoma progression

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ARTICLE INFO

Available online 17 July 2014

Keywords:
Autophagy
Mitophagy
Signaling
Cancer
Melanoma
Anticancer therapy

ABSTRACT

Autophagy, or self-eating, is the most extensively studied lysosomal degradation pathway for the recycling of obsolete or damaged cytoplasmic materials, including proteins and organelles. Although this pathway was initially thought to function as trafficking system for 'in bulk' degradation by the lysosomes of cytoplasmic material, it is now widely appreciated that cargo selection by the autophagic machinery is a major process underlying the cytoprotective or – possibly – pro-death functions ascribed to this catabolic process. Indeed increasing evidence suggests that in mammalian cells the removal of dysfunctional or aged mitochondria occurs through a selective degradation pathway known as 'mitophagy'. Due to the crucial role of mitochondria in energy metabolism, redox control and cell survival/death decision, deregulated mitophagy can potentially impact a variety of crucial cell autonomous and non-autonomous processes. Accumulating evidence indicates that during malignant transformation aggressive cancers hijack autophagy to preserve energy fitness and to acquire the plasticity required to adapt to the hostile microenvironment. However, whether and how mitophagy contributes to carcinogenesis, which pathways regulate this process in the cancer cells and how cancer cell-mitophagy impacts and modifies the tumor microenvironment and therapeutic responses, remain largely unanswered issues. In this review, we discuss novel paradigms and pathways regulating mitophagy in mammalian cells and the impact this process might have on one of the most dreadful human malignancies, melanoma.

1. Autophagy; basic molecular machinery

Macroautophagy (hereafter referred to as autophagy) is a regulated process of self-eating by which part of the cytoplasm, including long-lived or unfolded proteins and superfluous or damaged organelles, is trafficked through the aid of double-membraned vesicles, known as autophagosomes, to the lysosomes where cargo is degraded. Besides functioning as a key quality control mechanism crucial to control organelle number and fitness during homeostasis in all eukaryotic cells, recycling of intracellular components via this catabolic process supplies an alternative energy resource to survive in conditions of famine or reduced oxygen supply (Klionsky & Codogno, 2013). Although autophagy is constitutively active at basal levels sufficient to preserve homeostasis and cytoplasmic quality control, it can be stimulated 'on demand' whenever cells sense nutrient or oxidative stress, organellar damage or loss of proteostasis (Klionsky & Codogno, 2013). Stimulation of autophagy under these stress conditions is a pro-survival mechanism, although under certain circumstances autophagy may favor cell death (Marino et al., 2014).

Autophagy initiates by the formation of an isolation membrane (called phagophore), usually formed at the contact sites between the endoplasmic reticulum (ER) and mitochondria. Additionally other organelles, like the Golgi apparatus, post-Golgi compartments, as well as the plasma membrane may provide alternative sources (Rubinsztein et al., 2012), although the exact molecular players in phagophore-building and membrane trafficking in mammalian cells are only beginning to emerge (Bejarano et al., 2014; Zavodszky et al., 2013). The isolation membrane is further elongated to form double membrane vesicles, which deliver their cargo to the lysosomes for degradation and recycling (schematically presented in Fig. 1).

Abbreviations: AMPK, AMP-activated protein kinase; Atg, autophagy-related genes; BNIP3, BCL2 and adenovirus E1B 19 kDa-interacting protein 3; BNIP3L, BNIP3 like protein; CQ, chloroquine; Drp1, dynamin-1-like protein; ER, endoplasmic reticulum; Fis1, mitochondrial fission protein; FIP200, focal adhesion kinase family interacting protein of 200 kDa; HIF1, hypoxia-inducible factor 1; HKI, hexokinase I; IMM, inner mitochondrial membrane; MAMs, mitochondrion-associated membranes; Mff, mitochondrial fission factor; MPP, mitochondrial processing protease; mTORC1, mechanistic target of rapamycin complex 1; NIX, NIP3-like protein X; LC3, microtubule-associated light chain-3; LIR, LC3 interacting region; Mfn, mitochondrial outer membrane guanosine triphosphatase mitofusin; OMM, outer mitochondrial membrane; PARL, mitochondrial inner membrane rhomboid protease presenilin-associated rhomboid-like protein; PE, phosphatidylethanolamine; PGC-1α, transcription factor PPARγ co-activator 1α; PI3KC3, class III PI3K; PARKIN, *parkin* gene product; PINK1, phosphatase and tensin homologue (PTEN)-induced putative protein kinase 1; ROS, reactive oxygen species; TOM, the protein translocase of the outer mitochondrial membrane complex; TIM23, translocase of inner mitochondrial membrane 23 homologue; ULK1/2, Unc51-like kinase 1/2; VDAC, the voltage-dependent anion channel.

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http://dx.doi.org/10.1016/j.mito.2014.07.003
1567-7249/

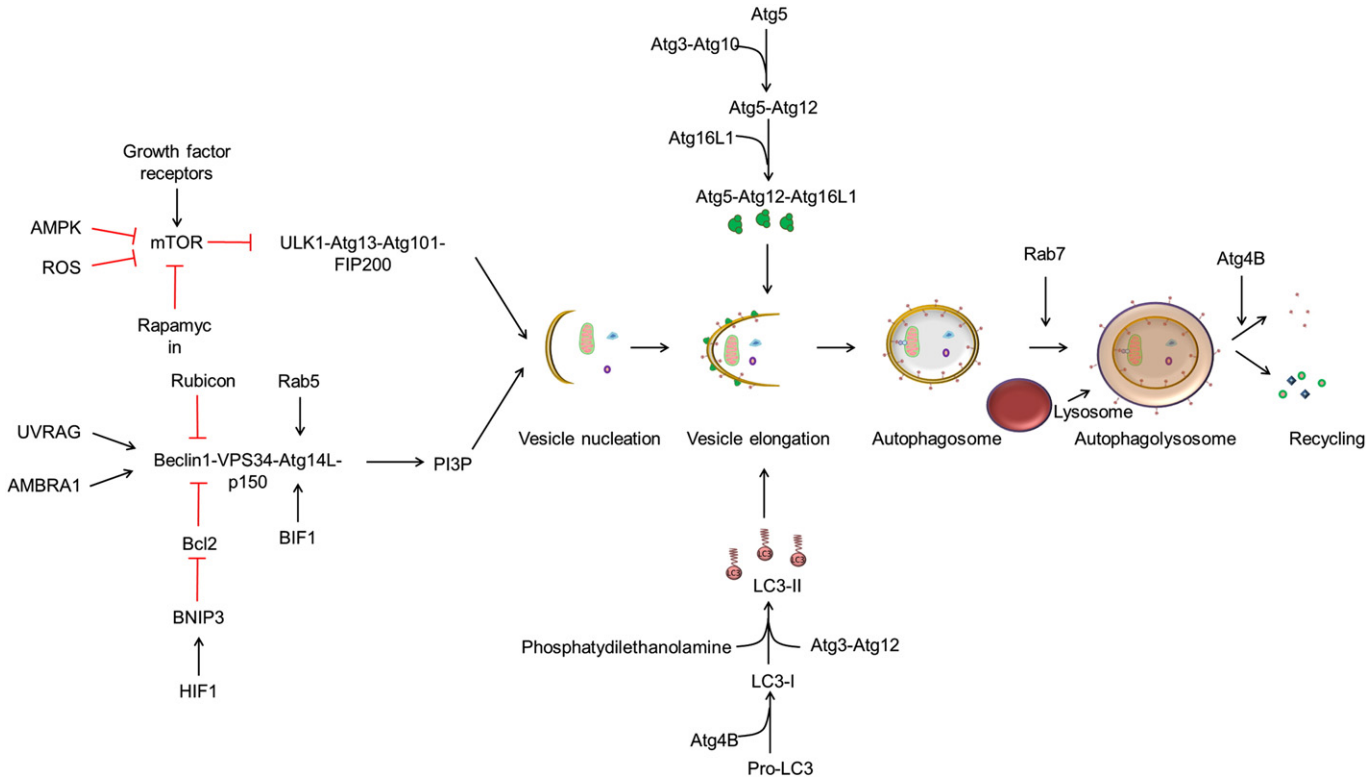

Fig. 1. Molecular signaling during autophagy. The figure schematically depicts the different stages of autophagy and their major molecular regulators. Vesicle nucleation, is controlled by the ULK1–Atg13–Atg101–FIP200 complex which is negatively regulated by the mTORC1 complex. Upon autophagy activation, mTORC1 dissociates from the ULK complex, leading to ULK1/2 and Atg13 dephosphorylation and translocation of the complex to the autophagosome formation site. Autophagy activation by reduced cellular energy occurs through the AMP-activated protein kinase (AMPK), which phosphorylates Raptor, hereby inhibiting mTOR. Upon activation, the ULK-complex is involved in the correct localization of the Beclin1–VPS34–Atg14L–P150 complex, whose activity is positively regulated by Bif-1, UVRAG, Rab5 and AMBRA1 or inhibited by Bcl2 and Rubicon. Upon autophagy activation, ULK1 phosphorylates Ambra1, releasing the PI3K3 complex from the cytoskeleton and enabling the relocalization of this complex to the ER. Vesicle elongation, is mediated by two-ubiquitin-like conjugation systems. Firstly, Atg5 conjugates to Atg12 assisted by Atg7 and Atg10 (E-1 and E-2 like enzymes, respectively). The Atg5–Atg12 conjugate binds to Atg16L forming the Atg16L complex, which transiently associates to the growing autophagosomes and leaves the autophagosomes after closure. The second ubiquitin-like conjugation system involves LC3 (microtubule-associated light chain-3), which is cleaved by the cysteine protease Atg4 to produce LC3-I. Its glycine residue conjugates to phosphatidylethanolamine (PE) with the assistance of Atg7, the E2-like enzyme Atg3 and the Atg16L complex to produce the lipidated autophagosomal-localized LC3-II. Autophagolysosome formation. Rab7 promotes microtubule transport and fusion of the autophagosomes with the lysosomes. Recycling. After autophagolysosome formation LC3-II located at the cytosolic surface of the autophagolysosome undergoes Atg4-mediated decoupling from PE to be recycled.

At the molecular level the different stages of autophagy are spatially and hierarchically controlled by a set of autophagy-related genes (Atg), conserved between yeasts and mammals (Chen & Klionsky, 2011; Wirawan et al., 2012). A key regulator of autophagy-initiation in mammalian cells is the Unc51-like kinase 1/2 (ULK1/2) complex, containing Atg13, Atg101 and FIP200 (focal adhesion kinase family interacting protein of 200 kDa). This complex is negatively controlled by the mechanistic target of rapamycin complex 1 (mTORC1) kinase. mTORC1 activation under nutrient/growth factor-rich conditions leads to phosphorylation and inactivation of ULK1/2 and Atg13. Starvation-induced activation of AMP-activated protein kinase (AMPK), reactive oxygen species (ROS), or rapamycin treatment causes the inactivation of mTORC1, which results in the induction of autophagy (Fig. 1). Upon activation, the ULK-complex is involved in correctly localizing the class III PI3K (PI3KC3)/Vps34 complex, containing p150, Atg14L and Beclin1, which regulates nucleation and assembly of the phagophore membrane. The activity of this complex is tightly controlled by positive regulators like UVRAG, AMBRA1, Rab5 and BIF1 and negative regulators like Rubicon (Fig. 1). Beclin 1 in this complex can bind – through agency of its BH3 domain – anti-apoptotic BCL2 family members, which can by this mechanism exert a negative control on autophagy induction. Upon activation ULK1 phosphorylates AMBRA1, hereby releasing the AMBRA1 containing PI3KC3-complex from the microtubules, resulting in its relocalization to the ER (Chen & Klionsky, 2011; Wirawan et al., 2012). Important signaling molecules for the regulation of the elongation, shaping, and sealing of the autophagosomal membrane are two-ubiquitin-like conjugation systems, regulating the formation of the Atg5–Atg12–Atg16L complex and the phosphatidylethanolamine (PE)-conjugation of the microtubule-associated light chain-3 (LC3), which becomes associated to the autophagosomes through the entire process serving as a marker for their identification (see Fig. 1 for a more extensive description).

Mature autophagosomes can merge with endocytic vesicles (early or late endosomes) to become amphisomes or directly fuse with lysosomes, where the cargo is degraded by lysosomal hydrolases. Fusion of the autophagosomes with the lysosomes is mediated by Rab7, which promotes microtubule transport and Syntaxin 17, a SNARE protein that is localized on mature autophagosomes (Kramer, 2013). After autophagolysosome formation building blocks are freed into the cytosol and LC3 located at the cytosolic surface of the autophagolysosome undergoes Atg4-mediated decoupling from PE to be recycled.

Because autophagy is usually activated by cellular stress that may concurrently initiate apoptotic cell death, accumulation of autophagosomes is often detected in cells committed to die. This has led to the suggestion that autophagy may be causative for cell death. However, the occurrence of the so called autophagic cell death, i.e. cell death caused by the autophagy genetic machinery, has been documented so far during developmental stages/tissues in lower organisms, whereas in mammalian cells appears to be limited to selective pathological and/or drug-induced conditions (for an extensive review see (Marino et al., 2014)).