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REVIEW

Autophagy as a target for hematological malignancy therapy

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ABSTRACT

Autophagy is an essential metabolic pathway by which the intracellular unwanted materials are digested within lysosomes for cellular homeostasis. It provides energy and building blocks upon starvation or other stresses. Autophagy even contributes to cell death especially under apoptosis incompetent conditions depending on the cellular contexts. Dysfunction of autophagy involves in the initiation and progression of multiple diseases and their treatments. But its principles and clinical applications have not been fully elucidated yet. Basal autophagy may serve as a tumor suppressive mechanism during tumorigenesis; nevertheless, excessive autophagy even works as a pro-survival pathway in already established cancers. Recently, mounting evidence highlighted its key roles in the genesis and therapy of various hematological malignancies. The combinations of autophagy inhibitors (such as chloroquine) with some first-line drugs, as well as novel autophagy-based manipulations, including Bcl-2 family regulation, caspase-dependent cleavage of ATG proteins and microRNA replacement are clinically or experimentally applied, representing promising approaches for their clinical treatments. This review is therefore to discuss the recent progress in autophagy machinery and its association with hematological malignancy therapy.

1. Introduction

Autophagy is an evolutionarily conserved, bulk digestion process in eukaryotes. It breaks down unwanted materials, releasing basic building blocks, such as amino acids, fatty acids and nucleotides for energy supply and macromolecule turnover. Different from the proteasomal degradative pathway, autophagy digests long-lived proteins and damaged or superfluous organelles by lysosomes [1]. Thus, autophagy maintains cell homeostasis through degradation and turnover of the intracellular components at a basal level. Under various stresses, cells undergo high levels of autophagy to adapt to the changing environment (e.g., nutrient starvation, oxidative stress, unfolded protein response, and ER stress), allowing cells to withstand the insults to prevent cell damage or death. Furthermore, if autophagy is stimulated up to a threshold at which normal cell functions are compromised, cells are inevitably committed to apoptosis or autophagic cell death.

Crucially, autophagy plays critical roles in the pathogenesis of diverse diseases, including inflammation, atherosclerosis, cardiovascular disease, neurodegeneration, and cancer [2]. As autophagy eliminates misfolded proteins and damaged organelles, the normal function of autophagy is critical for the maintenance of cell homeostasis and metabolism; impaired autophagy may lead to accumulation of misfolded proteins, mitochondria dysfunction, high levels of reactive oxygen species (ROS), DNA damage and genomic instability. These may be cytotoxic or lead to carcinogenesis and tumor progression. Moreover, with the activation of autophagy as a prosurvival mechanism, immortalizing cells sustain the stress stimuli, including nutrient insufficiency, hypoxia, chemotherapy and radiotherapy, reducing the effects of various therapeutic regimens. Thus, via enhancing the efficacy of pharmaceuticals or drugs, autophagy inhibition represents an effective therapeutic approach against some diseases, including hematological malignancy.

Autophagy can be divided into three types according to the patterns of cargo sequestration: macroautophagy, microautophagy and chaperone-mediated autophagy. Microautophagy digests cytoplasmic components directly by lysosome, and chaperone-mediated autophagy delivers only proteins that have a pentapeptide motif KFERQ in its amino acid sequence. Macroautophagy (hereafter referred to as autophagy) is distinct from the other two types by the characteristic structure, autophagosome. In the recent decade, roles of autophagy, as well as its molecular machinery have gained in-depth study. In this review, we sought to give insights into the association and causality of autophagy and hematologic malignancies hinting at potential therapeutic targets for blood tumors based on autophagy.

2. Autophagic machinery

The autophagic process includes multiple continuous steps: initiation, nucleation, elongation, closure of the cup, maturation and degradation. Till now, over 30 AuTophaGy-related (*Atg*) genes have been identified in yeast *Saccharomyces cerevisiae*, and their homologs were

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characterized in higher eukaryotes. These ATG proteins are hierarchically recruited to the autophagosome formation site for the boosting of the core autophagic machinery, which is strictly modulated by the upstream signaling pathways. Autophagic proteins involved in autophagosome formation assemble into several functional complexes: (1) UNC51-like kinase 1 (ULK1) complex; (2) class III phosphoinositide 3-kinase (PI3K) complex; (3) ATG9 recycling system; and (4) ubiquitin-like conjugation systems including ATG12–ATG5–ATG16 and microtubule-associated protein light chain 3 (LC3)-phosphatidylethanolamine (PE) conjugation system (Fig. 1) [3].

The ULK1 complex acts presumably the most upstream of conserved ATG proteins. It consists of ATG13, FIP200, ATG101 and ULK1. In nutrient-rich conditions, activated mammalian target of rapamycin complex1 (mTORC1) inhibits autophagy by associating with ULK1 complex and phosphorylating ULK1 and ATG13 [4]. Upon starvation, mTORC1 releases the ULK1 complex, causing modest dephosphorylation of ULK1 and ATG13 [5]. Distinct from Atg1 in yeast, ULK1 associates with ATG13 and FIP200 even under nutrient-rich conditions [6]. When activated, ULK1 provokes an autophosphorylation and phosphorylation of ATG13 and FIP200 (Fig. 2A) [5–8]. ER-located ULK1 complex recruits PI3K complex, PI3P-binding proteins (such as WIPI-1/2 and DFCP1), LC3–PE (LC3-II) and ATG12–ATG5–ATG16L complex, and promotes the genesis of pre-autophagosomal structure (Fig. 3, left panel). ULK1 mechanistically associates with some LC3 homologs, such as GATE16, GABARAP, and LC3 [9]. Accordingly, identification of autophagy relevant ULK1 substrates would allow fully understanding of the molecular mechanisms underlying autophagy.

Class III PI3K (PIK3C3/VPS34) generates PtdIns(3)P for autophagy activation. The class III PI3K complex is composed of PIK3C3, BECN1, and PIK3R4/p150, providing a PI(3)P-enriched domain for autophagosome formation (Fig. 2B). These core components bind different partners, including ATG14/ATG14L/Barkor, UVRAG (UV radiation resistance-associated gene) and Rubicon/KIAA0226. PIK3C3 mostly colocalizes with UVRAG under nutrient-rich conditions; ATG14 substitutes for a portion of UVRAG at the same C2 domain of PIK3C3 to initiate autophagy (Fig. 2B) [10]. BECN1 interacts with UVRAG or AMBRA1 (activating molecule in Beclin1-regulated autophagy) with its coiled-coil domain. BECN1 forms three major complexes during autophagosome formation: AMBRA1–BECN1–PIK3C3–p150, ATG14–BECN1–PIK3C3–p150, and UVRAG–BECN1–PIK3C3–p150. The PIK3C3 kinase-produced PI3P activates phagophore generation with the aid of double FYVE-domain containing protein 1 (DFCP1) [11] and the Atg8

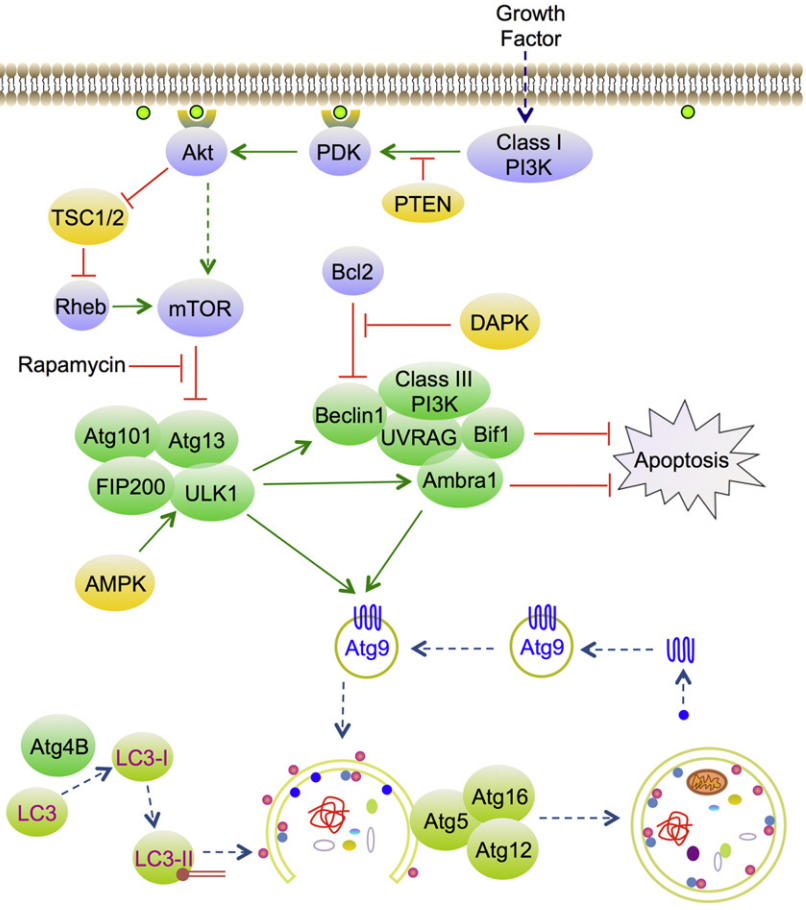

Fig. 1. Schematic overview of autophagosome formation. Class I PI3K generates PI(3,4,5)P3 when activated by the outer growth factors through the corresponding receptors. PI(3,4,5)P3 recruits PDK and Akt kinase to the plasma membrane where Akt stimulation occurs, leading to phosphorylation (P) of mTORC1. Tumor suppressor PTEN hydrolyzes PIP3 to form PI(3,4)P2, counteracting the activity of PI3K. Activated mTORC1 associates and prohibits the ULK1/2 complex by hyperphosphorylation of ATG13 and ULK1. Upon starvation or rapamycin treatment, mTORC1 activity is inhibited and dissociates from ULK1/2 complex. AMPK phosphorylates and activates ULK1/2 depending on the AMP/ATP level. Activity of class III PI3K complex, consisting of BECN1, p150, ATG14, AMBRA1 and PIK3C3/Vps34, is modulated by ULK1 through phosphorylation of BECN1 and AMBRA1. Robust AMBRA1 may restrain the cellular apoptosis, while caspase-dependent apoptosis promotes AMBRA1 degradation. Bcl-2 proteins associate with BECN1 at the BH3 domain, inhibiting autophagy. DAPK functions to dissociate Bcl-2 from BECN1 via phosphorylation of BECN1. ULK1/2 and PI3K complexes translocate to ER where omegasome is triggered to emerge. Bif1 facilitates membrane fission and ATG9 recycling when it forms a complex with PIK3C3 and BECN1 through UVRAG. Bif1 also impedes the apoptosis process, and promotes autophagic cell death. In mammalian cells, ATG9 mainly locates on the trans-Golgi network and endosomes. ATG9 transiently localizes to phagophore and non-phagophore site, contributing to transportation of lipid from donor sources to the autophagosome formation site. Two ubl protein conjugation systems, ATG12–ATG5–ATG16L and LC3–PE, are recruited to this site, facilitating expansion of the double membrane structure and forming autophagosome when enclosed. The matured autophagosome is transferred to MTOC through dynein motor proteins and fuses with lysosome for degradation.