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Autophagy: a lysosomal degradation process for cellular homeostasis and its relationship with oral squamous cell carcinoma

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Autophagy is an evolutionarily well-conserved cellular homeostasis program that responds to various cellular stresses and degrades unnecessary or harmful intracellular materials in lysosomes. Accumulating evidence has shown that autophagy dysfunction often results in various human pathophysiological conditions, including metabolic disorders, cancers, and neurodegenerative diseases. The discovery of an autophagy machinery protein network has revealed underlying molecular mechanisms of autophagy, and advances in the understanding of its regulatory mechanism have provided novel therapeutic targets for treating human diseases. Recently, reports have emerged on the involvement of autophagy in oral squamous cell carcinoma (OSCC). Although the role of autophagy in cancer therapy is controversial, the beneficial use of the induction of autophagic cell death in OSCC has drawn significant attention. In this review, the types of autophagy, mechanism of autophagosome biogenesis, and modulating molecules and therapeutic candidates affecting the induction of autophagic cell death in OSCC are briefly described.

Keywords: Autophagy, Therapeutic targets, Autophagosome biogenesis, Oral squamous cell carcinoma

Introduction

Autophagy is a lysosome-dependent catabolic pathway to remove intracellular materials, such as protein aggregates, long-lived/damaged organelles, and virus/bacteria [1]. Autophagy functions as a fundamental cellular homeostasis program to provide source of energy and building blocks, or to eliminate harmful/dysfunctional intracellular materials to cope with many different pathophysiological conditions. In this regard, autophagy is highlighted to closely associated with various human diseases, such as diabetics, cardiovascular diseases, neurodegenerative diseases, aging, and cancers [2-4].

Three Types of Autophagy

Autophagy is divided into three categories: microautophagy, chaperone-mediated autophagy (CMA), and macroautophagy, depending on how the autophagic substrates move to lysosome. At first, microautophagy is generally believed to be a non-selective autophagy process in which the substrates are directly recognized and enter the lysosome for degradation. It is mainly observed in yeast, but is also observed in mammals

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[5]. Second, CMA is a selective and unique autophagy process in mammals. CMA substrates are cytosolic unfolded protein aggregates that are transferred to lysosomes with HSP70 (heat shock 70 kDa). CMA substrates have a conserved CMA-targeting motif (KFERQ) for HSP70 interaction. The CMA-targeting motif undergoes a conformational change to expose and complex with HSP70 upon CMA signaling. The HSP70-CMA substrate complex is recognized by the lysosomal membrane receptor, Lysosome-Associated Membrane Protein type 2A (LAMP-2A), and then enters the lysosome [6-8]. Finally, macroautophagy (hereafter autophagy) uses a unique transport vesicle with a double-membrane structure, an autophagosome, to deliver its destructive cargos to the lysosome [9]. It could be a non-selective process, but accumulating reports have shown that it chooses and selectively degrades intracellular materials in response to a certain condition [9]. The autophagosome fuses with lysosome to form the autolysosome, where the cargos are eventually degraded by lyososomal acid hydrolases. Extensive genetic and biochemical studies in yeast and fly have provided a mechanistic insight into autophagy machinery proteins, such as autophagy-related gene (ATGs) family, vascular protein sorting (VPS) proteins, Rab small GTPases, and SNARE proteins [10,11].

Molecular Mechanism of Autophagosome Biogenesis

Autophagosome biogenesis is carried out by the coordinated actions of autophagy machinery proteins, of which Unc-51 like autophagy activating kinase 1 (ULK1) complex and VPS34 complex initiate autophagosome biogenesis and autophagosomal membrane nucleation [9-11]. In addition, two ubiquitin-like (UBL) conjugate systems, ATG12-ATG5-ATG16L1 and microtubule associated protein 1 light chain 3 (LC3) is required for phagophore (pre-autophagosomal structure) membrane elongation (Fig. 1) [12].

1. Initiation and nucleation: ULK1 and VPS34 complex

ULK1 is a serine/threonine kinase and orthologue of the yeast Atg1. ULK1 appears to be a most upstream regulator to trigger the autophagy program [11,13]. ULK1 forms a complex with FIP200, ATG13, and ATG101, which function to stabilize and activate ULK1, and also play an important role in targeting the ULK1 complex into the omegasome, a unique membrane structure initiating the phagophore formation [12,14]. ULK1 complex is regulated by various phosphorylations, especially

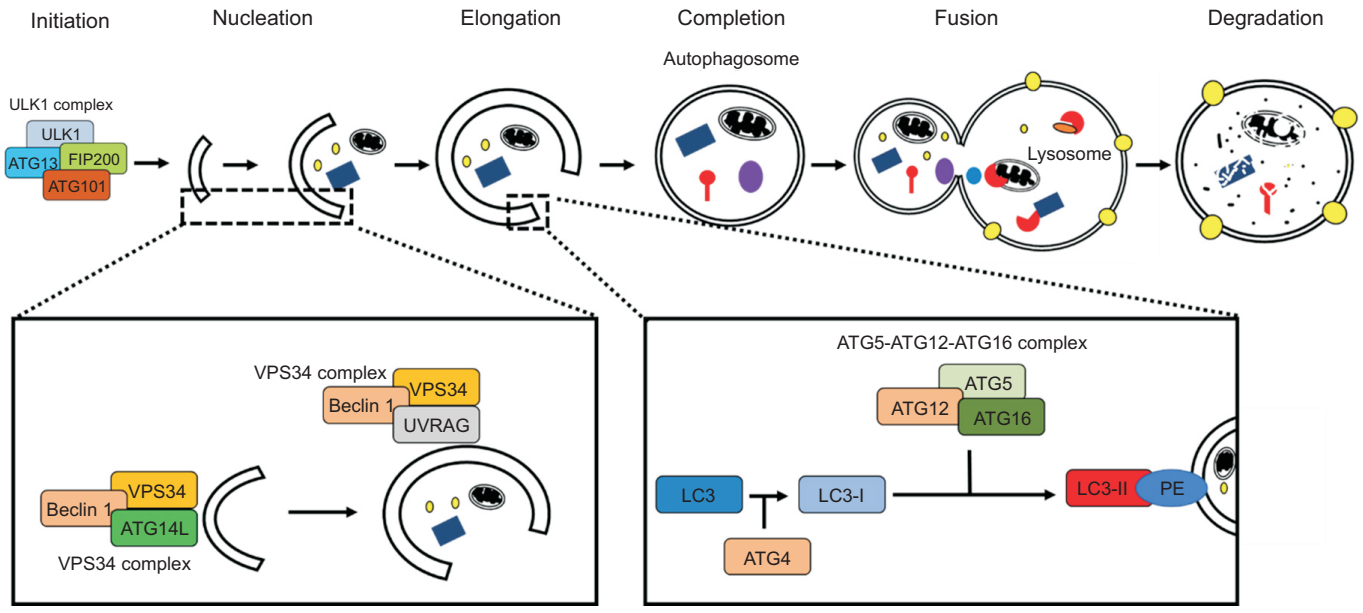

Fig. 1. Molecular mechanism of autophagy process. Autophagy occurs by a coordinated action of autophagy machinery proteins. ULK1 complex, consisting of the catalytic subunit ULK1 protein kinase and its associated-regulatory subunits such as ATG13, FIP200, and ATG101, initiates the phagophore formation by phosphorylating and activating VPS34 complex containing either ATG14L (PI3KC3-C1) or UVRAG (PI3KC3-C2), which in turn marks a distinct autophagosomal membrane with its phospholipid product, PI-3-P. ATG12-ATG5-ATG16L1 complex and phosphatidylethanolamine (PE)-conjugated LC3 (LC3-II) are recruited on this autophagosomal membrane for elongation and closure of the phagophore membrane. Closure of the phagophore membrane gives rise to a double-membrane bounded vesicle called the autophagosome, which matures and finally fuses with the lysosome to form the autolysosome. Revised from the article of Zhou et al. (Int J Biol Sci 2019;15:726-37) [45].

by mechanistic target of rapamycin complex 1 (mTORC1) and AMP-activated protein kinase (AMPK). Both mTORC1 and AMPK consist of key signaling pathways for cellular energy homeostasis, especially for amino acid and glucose metabolism [15,16]. mTORC1 inhibits the ULK1 complex by directly phosphorylating ULK1 to block ULK1 activation by AMPK [17]. However, once mTORC1 is inactive when the nutrient supply is limited, the mTORC1-dependent inhibitory phosphorylation is diminished, which allows AMPK-ULK1 interaction. AMPK directly phosphorylates to activate ULK1, leading to autophagy initiation [17,18]. Next, ULK1 stimulates VPS34 complex by phosphorylating a key component of VPS34 complex, Beclin 1 [19]. VPS34 complex is a class III phosphatidylinositol 3-kinase (PIK3C3/VPS34) to phosphorylate phosphatidylinositol (PI) on the endosomal membrane to generate PI-3-monophosphate (PI-3-P), a key membrane marker for autophagosome biogenesis [20]. VPS34 interacts with various proteins to form many different complexes that are responsible for numerous cellular functions such as the multi-vesicular body pathway, retrograde trafficking from endosomes to the Golgi, phagosome maturation, and autophagy [20], of which ATG14L and UVRAG (UV-radiation resistance-associated gene protein) makes the resulting VPS34 complex as a pro-autophagy complex [21]. In autophagy, VPS34 forms at least two distinct complexes, complex I with ATG14L (PI3K3-C1) and complex II with UVRAG (PI3K3-C2), which are required for different stages of the autophagy process [22,23]. The core complex unit contains a catalytic subunit VPS34 lipid kinase, a pseudokinase VPS15/p150, and a scaffolding subunit Beclin 1. Depending on the subcellular context, this core complex binds to either ATG14 or UVRAG in a mutually exclusive manner defining the PI3K3-C1 and -C2, respectively. The PI3K3-C1 containing ATG14 is necessary for the nucleation of the autophagosomal membrane and the UVRAG-containing PI3K3-C2 complex functions in autophagosome maturation and autolysosomal tubulation [24,25]. Notably, PI3K3-C2 also play a role in endosome trafficking and multi-vesicular body formation [26]. ATG14L targets PI3K3-C1 to the phagophore sites by its N-terminal cysteine-rich domain and the C-terminal amphipathic helix BATS (Barkor/ATG14L autophagosome targeting sequence) domain [27,28]. UVRAG regulates autophagosome maturation by binding to the HOPS (homotypic fusion and vacuole protein sorting) complex, which stimulates lysosomal fusion with the autophagosome [29]. A number of phospho-regulation mechanism has also been shown in the VPS34 complex. First, VPS34, Beclin 1, ATG14L, and UVRAG are all directly phosphorylated and regulated by AMPK-mTORC1 signaling. AMPK can phosphorylate both VPS34 and Beclin 1, but it appears to be regulated by ATG14L on the complex [30]. For instance, AMPK prefers to phosphorylate to inhibit VPS34 lipid kinase activity in non-autophagic VPS34 complex without ATG14L, whereas it phosphorylates Beclin 1 in PI3KC3-C1 or -C2 for activation. Notably, ATG14L is shown to be phosphorylated by mTORC1, which makes ATG14L-containing PI3KC3-C1 inactive [31]. Similarly, mTORC1 also phosphorylates UVRAG to inhibit the complex by recruiting the inhibitor protein RUBICON into the UVRAG-associated complex [24,25]. Upon amino acid starvation, which blunts mTORC1 signaling, mTORC1-dependent inhibitory UVRAG phosphorylation is diminished to release UVRAG from RUBICON, allowing UVRAG-HOPS complex interaction for autophagosome maturation with lysosome.

2. Elongation and completion of autophagosomal membrane: ATG5-ATG12-ATG16L and LC3 conjugation system

The elongation of autophagosomal membrane requires two UBL conjugation systems. First, ATG12-ATG5-ATG16L complex is prepared by a coordinated action of UBL proteins, in which ATG12 is covalently conjugated to ATG5 in a manner dependent on the E1-like activating enzyme ATG7 and the E2-like conjugating enzyme ATG10 [32-36]. ATG12-ATG5-ATG16L1 complex associates with the phagophore membrane for elongation and closure by recruiting LC3 on the phagophore membrane and promoting LC3 processing. The complex is dissociated upon the completion of autophagosome biogenesis [37,38]. The second UBL system is the LC3/ATG8 system. LC3 is first cleaved by the cysteine protease ATG4, exposing a C-terminal glycine (LC-I). It is further processed by ATG7 (E1-like), ATG3 (E2-like), and then conjugated to phosphatidylethanolamine (PE) with E3-like ATG12-ATG5-ATG16L1 complex [39,40]. In this sense, turnover of LC3-I into LC-II on immunoblotting is commonly used as an autophagy marker. LC3-II coordinates at phagophore membrane to elongation of the membrane and interacts with various autophagy receptors, such as p62/SQSTM-1 (sequestosome-1) and NDP52, which bind to the ubiquitinated cargos and then undergo oligomerization to deliver the autophagic substrates to the autophagosome [41-45].

Autophagy and Oral Cancer

Among oral cancers oral squamous cell carcinoma (OSCC) is a major type of head and neck carcinoma which accounts for about 90% of oral cancers. OSCC exhibits a survival rate of less than 60%. Diagnosis in late stage and lack of effective treatment make OSCC more significant disease [46-48]. OSCC showed many pathological differences from other cancers found in head and neck area [49]. However, the molecular and cellular mechanism of the pathogenesis are relatively not well understood. Recently, the relationship between oral cancers and autophagy has drawn attention [50]. Autophagy is cellular self-digestion pathway involved in many human disease and physiology [51]. There are controversial reports about the role of autophagy in cancer therapy: Autophagy may promote cell survival or induce cell death [52]. Therefore, understanding cytoprotective autophagy and autophagic cell death are important in development of effective therapeutic agents for OSCC. In this mini review, we focused on the molecules and chemicals involved in the induction of autophagic death of OSCC.

Current studies have shown that the relation of signaling/modulating molecules and the autophagy pathway [53-55]. Some of the molecules are involved in suppression and others are in progression of the autophagy pathway. Long noncoding RNA (lnc RNA) cancer susceptibility candidate 9 (*CASC9*) which is highly expressed in various cancers including OSCC [53]. Yang et al. [53] reported that depletion of CASC9 inhibited OSCC growth and autophagy-mediated cell death through the AKT/mTOR pathway.

Retinol-binding protein 1 (RBP1) is known to be involved in physiological functions, including in the pathogenesis of several types of cancer. Recently, the role of RBP1 in autophagy induction of OSCC was reported [54]. RBP1 activated autophagy through modulation of cytoskeleton-associated protein 4/p63 (CKAP4/p63), a type II transmembrane protein that functions as a receptor for several ligands, including anti-proliferating factor [54, 56].

Overexpression of secretory clusterin (sCLU) promoted autophagy through AMPK/Akt/mTOR signaling pathway. However, interestingly the induction of autophagy by sCLU resulted in cell survival and protection from apoptosis in oral cancer [55].

Chemicals and Natural Products Involved in Autophagic Cell Death of OSCC

Research on the development of chemicals and natural products which induce the autophagy pathway in OSCC are drawn attention in these days. A combination of proteinase inhibitor, bortezomib and irradiation treatment on human OSCC cells induced autophagic cell death through tumor necrosis factor receptor-associated factor 6 (TRAF-6) ubiquitination and TRAF6-medicated Akt activation. The authors suggested the possibility of novel strategy of OSCC treatment [57]. It was reported that chlorpromazine (CPZ) which is used to treat psychiatric disorders induced autophagy in oral cancer cells evidenced by autophagosome formation, expression of the related proteins and activation of the PI3K/Akt/mTOR/p70S6K pathway [58]. These results suggested that CPZ can be a novel treatment of oral cancer.

The use of natural products in treatment of oral cancer has been tried. One of the examples is nimbolide, a limonoid from the neem tree (*Azadirachta indica*) which induced both apoptosis and autophagy in oral cancer cells. Nimbolide enhanced apoptosis by overcoming the cytoprotective effect of autophagy [59]. Another natural product (phytochemical) ursolic acid is reported to induce apoptosis and autophagy in OSCC cells. Ursolic acid revealed LC3B-II conversion, enhanced p62 expression and autophagosome accumulation in OSCC cells [60]. 16-hydroxycleroda-3, 13-dine-15, 16-olide (HCD) isolated from a medicinal plant *Polyalthia longifolia* showed autophagy induction effect on human OSCC cells through LC3-mediated LC3-I/LC3-II/p62 pathway [61]. This compound has been shown to have autophagy induction effect on brain tumor cells evidenced by the increase in the autophagic markers including LC3-II and Beclin-1 [62] and apoptosis induction effect on human renal carcinoma cells through Akt, mTOR, and MEK-ERK pathways [63].

Conclusions

The role of autophagy in cancer is controversial because either cell death can be induced, or cell survival can be promoted by the autophagy pathway. There are a lot to be studied and discovered about this complex pathway and its relationship with oral cancer [50,64,65]. We briefly reviewed the types and mechanism of autophagy, and molecules and chemicals involved in the induction of autophagic cell death in OSCC. The understanding of the detailed mechanism behind autophagy will open a new passage to the prevention and therapeutics of oral cancers.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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