Review

Cross-talk between apoptosis and autophagy in lung epithelial cell death

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Abstract: As an essential organ for gas exchange, the lungs are constantly exposed to the external environment and are simulated by toxicants and pathogens. The integrity of lung epithelium and epithelial cells is crucial for fulfilling the physiological functions of the lung. The homeostasis of lung epithelial cells is maintained by a complex network by which survival and death are tightly regulated. Upon noxious stimulation, lung epithelium attempts to maintain its normal structure and function. Savage of injured cells and clearance of unsalvageable dying cells or unwanted proliferated cells constantly occur in the lung epithelium. Apoptosis, or programmed cell death, serves as a primary mechanism to discard unsalvageable cells or unwanted overgrowth. Autophagy, on the other hand, initially attempts to save and repair the injured cells. However, when the noxious stimulation is too strong and cell survival becomes unfeasible, autophagy behaves oppositely and cooperates with apoptosis, subsequently accelerates cell death. The imbalance between autophagy and apoptosis potentially leads to tumorigenesis or devastating cell death/lung injury. Therefore, the cross-talk between apoptosis and autophagy in lung epithelial cells is critical in determining the fate of epithelial cells and its balance of death/survival in response to environmental stimuli. In this review, we will focus on the current understandings of the communications between apoptosis and autophagy in lung epithelial cells. We will review multiple key regulators and their underlying mechanisms involved in the cross-talk between apoptosis and autophagy. The autophagic factors, such as the Beclin-1, ATG5, Fap-1, p62 and concentration-dependent LC3B, all closely interact with multiple apoptosis pathways. Understanding these regulations of apoptosis / autophagy cross-talk potentially provides novel targets for developing diagnostic and therapeutic strategies for many lung diseases, including lung injuries and malignancies.

Keywords: apoptosis, epithelial cell, autophagy, lung TBC1D4

Lung is an essential organ for gas exchange. This organ provides the imperative site for O₂ absorption needed for oxidative phosphorylation and CO₂ release required for pH regulation. Similarly, toxic gaseous bi-products are exchanged out of the system. The lung epithelium constantly interacts with noxious substances, infectious pathogens, and other gaseous compounds resulting in mixed and intertwining biochemical signals for both cell death and survival. In fact, even in the absence of noxious stimuli, lung epithelial cells undergo constant “proliferation-death” cycle to maintain the integrity of lung structure and gas-exchange function. Aged and/or injured cells undergo “suicidal” death under tightly controlled mechanisms to avoid a burst of secreted deleterious cytokines and molecules that will result in devastating injury to the adjacent cells/structures. This “low-grade” cell death constantly occurs in the lung parenchyma under healthy conditions. Strong noxious stimuli can induce a rapid cellular collapse leading to necrosis, an unsalvageable cell death. During this process, the cell loses membrane integrity abruptly and abruptly releases its cellular content (many of them are pro-inflammatory factors) into the extracellular space. On the other hand, the naturally occurring apoptosis
follows tightly regulated signal pathways into cellular breakdown [1].

As mentioned above, often times, the cell attempts to save and repair themselves from ambiguous incoming stimuli. In these cases, the cell monitors cellular stress via two well-studied mechanisms of regulated cell death, autophagy and apoptosis. Currently, how a cell chooses either apoptosis or autophagy as its major pathway remains unclear. However, accumulating evidence suggest that there is a cross-talk between these two pathways [2]. Autophagy and apoptosis share some common upstream regulating factors and the decision between the two cellular responses is the result of either interactive inhibition or stimulation between these two pathways.

**Brief introduction of apoptosis and autophagy**

Two well-established mechanisms for organized cell death include apoptosis (programmed cell death) and autophagy (cellular self-digestion) (Fig. 1). Portion of the enzymatic components in these two processes interacts, or cross-talks, with one another leading toward a potential decision for cellular destruction. Apoptosis, commonly known as programmed cell death (PCD), is a physiological cellular suicide process that is crucial for maintenance of physiological homeostasis. Apoptosis can be triggered in various manners, but ultimately involves either a caspase-dependent or caspase-independent signaling cascade resulting in the activation of biochemical catabolic enzymes and degradation of cellular organelles. Apoptosis is characterized by the collapse of cytoskeleton, condensation of cytoplasm, the dissociation of nucleus, and the formation of apoptotic bodies [3-5] (Fig. 1, Panel B). Apoptosis can be activated either intrinsically or extrinsically. For the intrinsic pathway, internal stress (such as heat, hypoxia, and nutrient deprivation) induces the release of small mitochondrial-derived activator of caspases (SMACs) from the mitochondria deactivating inhibitor of apoptosis proteins (IAPs) [5]. Cytochrome c is, then, released from the mitochondria via the newly translated mitochondrial

![Fig. 1. Panel A. Cellular death by autophagy, self-eating, initiates with the sequestering of cytoplasmic material by isolated double membranes named phagophores forming autophagosomes, double-membraned vesicles. In this cellular process, physiological stress (e.g., hyperoxia, CO exposure) could potentially initiate this cellular pathway in a normal cell (see Fig 1, step 1). Upon exposure to stress, the cell triggers the formation of phagophore, an isolated membrane, engulfing the surrounding biomolecules and organelles (step 2). The formed vesicle elongates and sequesters the biomolecules and organelles inside the lipid bilayer vesicle, autophagosome (step 3). The catabolically active organelle, lysosome, fuses with autophagosome forming autolysosome for lysosome-mediated enzymatic degradation of luminal macromolecules and the inner membrane inside the autophagic vacuole (step 4, step 5). During cellular death, majority of cellular content will be degraded (step 6). In contrast to apoptosis, autophagy is characterized by formation of autophagic vacuoles, retain of cytoplasmic cytoskeleton structures, and preservation of chromatins. Panel B. Apoptosis, self-killing, initiates with cytoskeleton reorganization causing cellular shrinkage and chromatin condensation (step 1, step 2). Furthermore, endonucleases degrade the nuclear DNA and protease hydrolyzes intercellular protein (step 3). The cytoplasm continues to collapse and slowly condenses into individual compartments (step 4). Lastly, the condensed compartments fragment forming cell blebs (step 5). These apoptotic bodies are taken up by another cell via phagocytosis for lysosome-mediated hydrolysis of macromolecules. Unique characteristics associated with this process include the collapse of cytoskeleton, the dissociation of the nuclease, and the formation of the apoptotic bodies.**
apoptosis-induced channel (MAC). The Cytochrome c then binds with both apoptotic protease activating factor-1 (Apaf-1) and pro-Caspase 9 to form apoptosome [6, 7]. This complex ultimately and indirectly activates Caspase-3 leading into cell death (Fig. 2). The extrinsic pathway is activated via signals and stimuli from outside the cell. A stimulus activates the death receptor causing the recruitment of Fas-associated protein with death domain (FADD) adaptors. The FADD activates the caspase cascade leading itself toward cellular destruction [8].

Autophagy, first reported in 1957, is a process that breaks down and recycles unnecessary proteins and organelles in cells [9, 10]. Upon stimulation, the cytosol and organelle are sequestered by a double membrane autophagosome. This vesicle, once matures, fuses with lysosome to form autolysosome for unspecific macromolecule degradation by lysosomal catalytic enzymes [11]. This process constitutes a common mechanism for cells to eliminate damaged organelles, destroy unwanted pathogens, recycle unused biomolecule or reorganize cellular nutrients during starvation. Though this process can be beneficial for the cell, however under certain cellular settings, excessive autophagy can serve as an alternative pathway for cellular death [11, 12]. In contrast to apoptosis, a cell undergoing autophagic cell death (type II cell death) retains its cytoskeleton structures, preserves its chromatins, and hydrolyzes its DNA (if occurs) very late in the system [2-4]. Autophagy has been characterized visually by the formation and retention of autophagic vacuoles in dying cells [12] (Fig. 1, Panel A). This cell death mechanism has been proposed to be induced by excessive influx of autophagy signaling [2, 13]. Depending on cellular factors (such as environmental factors and cell type), autophagy can act as either a pro-survival factor or cellular degradation response [13] (Fig. 3).

**Inducers of apoptosis and/or autophagy in lung epithelial cells**

As for lung epithelial cells, given their unique location and their interactions with numerous environmental molecules, the inducers of autophagy and apoptosis can be versatile. Unlike the typical inducers of autophagy and apoptosis (e.g. CO, hyperoxia), some uncommon inducers of autophagy and apoptosis were recently found. One of these inducers is an industrial produced multi-wall carbon nanotubes (MWCNT) [14]. HTT2800, a purified and low iron-concentrated form of MWCNT is exposed to lung epithelial cells (BEAS-2B) and induces autophagic activity in those cells. Under the same condition, Z-VAD-FMK (a caspase inhibitor) fails to prevent cellular death suggesting that this mechanism is not due to apoptosis [15]. Similarly, during the treatment of 3-MA (an inhibitor of autophagosome formation and LC3B expression), Caspase-3 is rapidly cleaved causing the activation of apoptosis [15]. Overall, the inhibition of autophagy triggers the activation of apoptotic activity indicating the existence of cross-talk between these two mechanisms.

Selenium, an essential nutrient often found in soil, is known to have anticancer properties, including repairing of DNA and reducing metastasis [16]. This element, particularly sodium selenite, is known to both trigger reactive oxygen species (ROS)-mediated apoptosis and promote autophagy [16]. Treatment of sodium selenium to A549 epithelial cells triggers both the intrinsic and extrinsic apoptosis, as well as the autophagic vacuoles formation [16]. This anti-tumor behavior is triggered and regulated by the generation of ROS in the sodium selenite-induced lung epithelial cells causing the collapse of mitochondria [16]. Pre-treatment with the autophagic inhibitor, bafilomycin, enhances apoptotic cellular death in sodium selenite-induced A549 cells. This suggests that autophagy could potentially act as a protective mechanism against apoptosis.

Celastrol, a compound extracted from root of *Tripterygium Wildordi*, exhibits anti-inflammatory, anti-neurodegenerative, and anti-cancerous activities. Celastrol also induces both autophagic and apoptotic activities in alveolar basal epithelial cells [17]. Another inducer of both autophagy and apoptosis is a mixture named water-accommodated fractions (WAF). WAF is mixture of volatile fraction of spilled oil and water due to chemical properties of added dispersants. Both apoptosis and autophagy occur in WAF-induced A549 lung [18]. Additionally, in A549 cell depleted with dynamin-related protein -1, there is a both decrease in basal autophagic activities and a reduction of apoptotic Cytochrome c release under apoptotic stimuli [19].

This mutual regulation in both autophagy and apoptosis activities are also demonstrated in infectious model. During the *in vitro* study on the infection of *Mycobacterium tuberculosis* (Tb) in human alveolar A549 epithelial cell, autophagic marker (LC3) expression significantly increases [20]. Furthermore, inhibition of the autophagic pathway magnifies the apoptotic activities in TB-induced A549 cells [20]. This correlation can be explained by a common factor that regulates both of these two events in the intrinsic apoptotic pathway. These outcomes imply and suggest a potential communication or “cross-talk” between the pathway of autophagy and apoptosis (Fig. 4).

**Key regulators involved in autophagy-apoptosis cross-talk in lung epithelial cells**

**LC3B**

In this recent decade, a marker typically used to characterize and measure autophagy’s progression is the Microtubule-Associated Protein 1 Light Chain 3 (LC3). Upon the induction of an autophagic signal, LC3-I is post-translationally modified into LC3-II by conjugating with phosphatidylethanolamines at the C-terminal glycine [21, 22]. The processed molecule is crucial for the regulation of the movement of the isolated double-membranad
phagophore, which overall dictates the extension and closing of phagophore forming an autophagosome [22]. For this reason, Lipedated LC3 or LC3-II is useful for detecting autophagy flux. After forming autolysosome, the luminal LC3-II is degraded; the LC3-II on the outer membrane is cleaved by autophagy related protein 4, ATG4, and are released into the cytosol [23, 24]. In studies on autophagy, LC3 isoform, LC3B, has been viewed to be a solid indicator and marker to measure the autophagy flux in a cell especially in its lipilated form [25]. Recent study has indicated that another LC3 isoform, LC3A-II, has possibly much greater correlation with autophagy flux than LC3B-II in mice tissues. The anti-LC3A antibodies have a greater specificity and detectability for LC3A-II than the respective anti-LC3B antibody for LC3-II in mice tissues [24].

LC3B’s accurate identification of autophagy is due to its crucial role in this pathway. This protein has been commonly viewed as having crucial anti-apoptotic effects. In many instances under stressful stimulus, autophagic indicator LC3B is over-expressed to prevent cell death, especially apoptosis. Over-expression of LC3B in sepsis-induced lung injury in mice has shown to increase survivability, decrease inflammation in lung, and decrease in cell death [26]. However, in many scenarios, after exceeding a certain expression level of LC3B, LC3B tends to lose its cytoprotective effect and triggers cellular suicide; lung epithelial cells that are overexpressing LC3B are more prone to cigarette smoke-induced cellular death by apoptosis than normal lung epithelial cells [27]. In a sense, autophagy provides the cells, including lung cells, an opportunity for adaption before fully committing to apoptosis. However, too much LC3B would trigger apoptosis.

Cross-talks between LC3B and the components of apoptotic pathway

LC3B has recently been found to have interactions with apoptotic proteins. LC3B forms a multimeric complex with Cav-1 and Fas (Fig. 5, Panel A). Cav-1 regulates both the autophagic factor, LC3B, and apoptotic factor, Fas, by

**Fig. 2.** Apoptosis, programmed cellular death, can be triggered through two pathways, intrinsic or extrinsic apoptotic pathway. The intrinsic (mitochondrial mediated) pathway (left) is characterized by the release of Cytochrome c from the mitochondria triggering the formation of apoptosome (not shown) and leading toward cellular death. The extrinsic (death receptor mediated) pathway is characterized by the activation through the binding of pro-apoptotic factors onto the Fas receptor forming a DISC complex. The formation of this complex, in return, initiates the caspase cascade (not shown) and, eventually, cell death.
binding with them on different domains [27]. It has been recently known that Cav-1 knockdown mice express a greater apoptotic activity and autophagic activity than wild-type mice under cigarette smoke (CS) [28] (Fig. 5, Panel C). This implies Cav-1’s functional role in down-regulating autophagy and apoptosis under CS-induced stress [28]. Additionally, in LC3B knockout mice under the presence of chronic CS exposure, the lung epithelial cells undergo a decreased apoptotic activity compared to the lung cells of wild type mice [13, 27]. This suggests that LC3B up-regulates apoptotic cellular death in this environment. The trimeric LC3B-Cav-1-Fas complex serves as an anti-apoptotic factor at a basal level. Due to the anti-apoptotic nature of this complex, it is hypothesized that cigarette smoke extract (CSE) treatment causes the dissociation of Cav-1-Fas-LC3B complex and the assembly of DISC [27, 28] (Fig. 5, Panel B). In the case of the LC3B knockout mice, the lung cell was not able to express LC3B to separate the heterotrimeric/heterodimeric (Cav-1-Fas) complex preventing DISC formation, and apoptosis (Fig. 5, Panel D). Due to these observations, it is hypothesized that LC3B acts as a pro-apoptotic factor by causing the disassociation of Cav-1-Fas complex and initiating the activation of DISC formation during extreme cellular stress [27].

Many inducers can activate autophagy activity and if not physiologically controlled may lead to cell death \textit{via} apoptosis or autophagy. In most cases, this cellular behavior is related to stress in endoplasmic reticulum (ER). Majority of the time during ER stress, either caused by external inducers (e.g. cigarette smoke, excessive oxygen) or unfolded protein response (UPR), it causes an increase in autophagic protein expression [29, 30]. Two of the well-studied inducers that can potentially activate both autophagic and apoptotic protein expression are the elevation of the reactive oxygen species (ROS) due to excess oxygen and the induction by hydrogen peroxide (H$_2$O$_2$) [31].

This generation of ROS due to a high oxygen concentration (hyperoxia) can induce apoptosis in lung epithelial cells [32], ultimately causing acute lung injuries. ROS generation increases the autophagic marker, LC3B, initiates the degradation of damaged organelles and seizes the cell from immediate apoptosis. As for the molecular mechanism, the LC3B interferes with Fas-DISC formation at the lipid raft and eventually inhibits the subsequent downstream caspase cascade and apoptosis [32]. The interaction, as mentioned earlier, requires caveolin-1 to bind with LC3B and Fas to form a multimeric complex [31]. The LC3B-Cav-1-Fas blocks Fas interaction with DISC inhibiting the activation of apoptosis. To further support this, siRNA directed knockdown of LC3B leads to the activation of hyperoxia-induced apoptosis in lung epithelial cells [33]. In this case, the lack of LC3B prevents DISC formation. Similarly, the overexpression of LC3B protects the lung epithelial cell from cellular death after hyperoxia [33].

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{autophagy_diagram.png}
\caption{The balance of autophagy, “self-eating”, and proliferation, “cellular growth” leads to a balanced cell. Greater proliferation leads to rapid growth in the cell and greater autophagy leads to cellular death.}
\end{figure}
ATG5/12

Another biomarker used for detecting autophagy activity and signaling is the autophagy-related 5 (ATG5) and its formation of ATG5-ATG12 complex. In response to cellular stress, ATG5 forms a complex with ATG12 to trigger the formation of LC3-II by the conjugation of LC3-I and phosphatidylethanolamine [21, 34].

Cross-talks between ATG 5/12 and the components of apoptotic pathway

It has been reported that the knockdown of ATG5, a pro-autophagic factor needed for the extension of phagophore, blocks autophagic vacuole formation and inhibits cellular death [35]. Interestingly, the over-expression of the same ATG5 increases vacuole formation and expresses some apoptotic characteristics including cell shrinkage and nuclear condensation. However, the nuclear condensation does not display any fragmentation [35]. Furthermore, it has been identified that this molecule binds at the death domain of the extrinsic pro-apoptotic factor, FADD [35]. The physical interaction between ATG5 and FADD in lung cells causes autophagic cellular death or type II cellular death [35]. This implies that ATG5 and FADD contribute to the regulation of both apoptosis and autophagy pathways. Without a doubt, in lung epithelial cells, one of the future directions of research is to explore extensively the cross-talk between ATG5/12 and apoptosis. In fact, studies using other cells have suggested that cleavage of ATG5 play an essential role in mediating apoptosis [36].

Sharing common functions as LC3, autophagic deficiency or siRNA directed knockdown of ATG5 increases the cleavage and activity of Caspase-3 in pancreatic β-cells [37]. Similarly, in osteoarthritis study, the inhibition of ATG5 via siRNA directed knockdown increases the amount of cleaved Caspase-9 and apoptotic behaviors. Conversely, an increase in autophagic factors reduces cleavage of Caspase-9 and apoptosis [38]. This reciprocal relationship between ATG5 factors and caspases shows a deeply enrooted cross-talk between autophagy and apoptosis.

The combination of pro-apoptotic factors, Bax and Bak, serves as anti-autophagic factors. Bax and Bak trigger the mitochondrial outer membrane permeability in the apoptotic pathway. Under an etoposide treatment or a constitutively expression of pro-apoptotic factors Bim and Bad in double knockdown Bax/Bak mouse embryonic fibroblasts (MEF), the cells fail to trigger apoptosis but undergo a delayed autophagic death, expressing the accumulation of apoptotic factors, ATG5-ATG12 complex.

![Fig. 4. Hyperoxia, Sodium Selenium, Celastrol, Water accommodated fractions (WAF), and Tb infection can induce either apoptosis or autophagy. The enzymes that regulate autophagy (Beclin-1 and LC3B) can suppress apoptosis promoting cellular survival. Outside factors, HO-1, can also inhibit apoptosis; HO-1 can either promote or inhibit autophagy depending on the cell type and concentration. Autophagy can either lead to cellular survival or cellular death (type II cell death) depending on cellular factors.](http://www.researchpub.org/journal/jbpr/jbpr.html)
and Beclin-1 [39, 40]. However, other apoptotic repressors (Apaf-1 knockout, Caspase-9 knockout and caspase inhibitor Z-VAD) fail to induce autophagy with etoposide treatment, suggesting that the combination of apoptotic factors Bax/Bak has regulatory function in autophagy pathway [39]. This phenomena suggests that Bax/Bak could possibly indirectly suppress autophagy to activate apoptosis.

In addition to ATG5-ATG12 complex regulation, truncated ATG5 has been indicated to interact with Bcl-xL, a pro-survival Bcl-2 class family protein, to promote apoptosis [41]. Similar to ATG5, it has been noted that ATG12, containing BH3-like motif, non-covalently interacts with anti-apoptotic Bcl-2 family to inactivate Bcl-2’s anti-apoptotic effects to progress and promote apoptosis [42]. The siRNA directed knockdown of ATG12 is shown to be able to inhibit mitochondrial Cytochrome c release.

It has been detected that autophagic factors activate NFκB, a factor for anti-apoptotic pathway. Without these autophagic factors, NFκB activation is compromised. Deletion of ATG5, ATG7 or Beclin-1 using siRNA-directed silencing inhibits the activation of this crucial pro-survival factor [43].

Beclin-1

Cross-talks between Beclin-1 and the components of apoptotic pathway

Beclin-1, another major factor required for the activation of autophagy, has shown to influence apoptosis activities. Recently, it has been shown that siRNA directed knockdown of Beclin-1 inhibits DISC formation when induced by the pro-apoptotic factors [44]. Therefore, Beclin-1 is another autophagic regulator, sharing similar behavior as LC3B, which inhibits DISC formation (Fig. 4). It is known that the siRNA knockdown of either Beclin-1 or LC3B reduces the activation of the caspase cascade, particularly the cleavage of Caspase 8 and 3 [45]. However, these two pathways are believed to be separate.

Beclin-1 molecular pathway is one of the surviving methods in which drives the survival of cells from apoptosis. This protein is first found as a binding partner to anti-apoptotic Bcl-2 family, such as Bcl-2, Bcl-xL, and Mcl-1 [46]. Later, it is established that the over-expression of Beclin-1 suppresses apoptotic activity [47]. However, the dissociation of Beclin-1 and Bcl-1 is essential for the suppression of apoptotic activity. For instance, during stress, Beclin-1 dissociates from Beclin-1-Bcl2 complex and inhibits Caspase-3 activation. However, during excessively prolonged stress and expression of amplified amount of caspase, Beclin-1 is cleaved causing a seize in autophagy and progression toward apoptosis [48]. The Bcl2-dependent feedback loop causes a delay between autophagy and apoptosis under various stress levels [48].

Beclin-1’s interaction with Bcl-2, a cellular anti-apoptotic factor, which reduces autophagy can be explained via Bcl-2’s structural pattern. Bcl-2 has a leucine-rich nuclear export signal (NES) motif which is crucial for the activation of autophagy; a mutation in the sequence interferes with the promotion autophagy during nutrient-deprivation [49]. Just upstream of the NES motif, Beclin-1 has a binding region for both pro-survival and anti-apoptotic factors Bcl-2 and Bcl-xL [50]. Among the three structure domains (BH3 domain, central coiled-coil domain, and evolutionarily conserved domain), the BH3 interactions with Bcl-2 and Bcl-xL [51]. The binding of Bcl-2 and/or Bcl-xL on Beclin-1 inhibits Beclin-1’s autophagic activity [51]. As predicted, a mutation in this BH3 domain disrupts the interaction between Beclin-1- Bcl2 complexes and induces autophagy [51, 52]. Furthermore, pro-apoptotic BH3-related factors (Bad, Noxa, and Bik) inhibit the interaction between Beclin 1– Bcl2 complexes and activate autophagy [51, 53]. Thus, these apoptotic factors can induce both pro-autophagic and anti-autophagic activities. It is, however, unsure if this activity is exhibited in lung epithelial cells. Verifications on the role of Beclin-1 in the cross-talk between apoptosis and autophagy require further studies.

Importantly, the cross-talk between apoptosis and autophagy also occurs upstream of Beclin-1. The upstream factor of the autophagic activator, Beclin-1, is further regulated by apoptotic factors. The activating molecule in Beclin-1-regulated Autophagy (AMBRA1) is regulated by caspase. It is demonstrated that AMBRA1 can be cleaved by caspase at D482 site [54]. The cleavage of AMBRA1 by caspases or siRNA directed knockdown of AMBRA1 causes the progression of apoptosis [54]. Conversely, the over-expression of AMBRA1 prolongs autophagy. It is deduced by Pagliartini et al. that AMBRA1 must be degraded to completely seize autophagy for apoptotic progression.

Additionally, it has been demonstrated that siRNA knockdown of Beclin-1 suppresses survivin (an inhibitor of apoptosis) activity and causes increased sensitivity to tumor necrosis factor-related apoptosis-inducing ligand [55]. This association and self-regulation displays a cross-talk between anti-apoptotic factors and autophagic activation factors.

HO-1 and CO

Cross-talks between autophagic gene products and cytoprotective molecules

Heme oxygenase-1 (HO-1) is an enzyme that catabolizes heme into carbon monoxide (CO), free iron, and biliverdin [56]. This molecule is predicted to be the preliminary response against excessively ROS due to high oxygen stress; the subsequently produced carbon monoxide is also in part of this cytoprotective pathway against oxidative stress [56, 57]. Under a low concentration of apoptotic promoting reagent, cigarette smoke extract, the up-regulation of HO-1 promotes cellular survival by down-regulating both apoptotic (DISC formation) and autophagic signaling [45, 57]. Furthermore, HO-1 overexpression by
adenovirus vector down-regulates both apoptotic and autophagic activities in CSE-treated bronchial epithelial cells; this includes the inhibition of LC3 processing and blockage of DISC formation [45]. It has been also reported that HO-1 silenced lung endothelial cells have a decreased LC3B at baseline than non-silenced endothelial cells [58]. Additionally, these silenced cells inhibit the activation of autophagy and exhibit apoptosis when induced by hyperoxia [58]. However under a different stimulus, namely CSE-treatment, the deletion of HO-1 increases LC3B activity in the bronchial epithelial cells. Similarly to exposure under hyperoxia, cells exposed to CSE exhibit apoptosis [45]. Although the mechanism remains unclear, HO-1 is definitely a much needed factor for the regulation of apoptosis and autophagy (Fig. 4).

The same is true for carbon monoxide (CO), which acts down-stream of HO-1. In the apoptotic pathway, CO promotes anti-apoptotic factors, inhibits the activation of caspase cascade, and prevents apoptosis [56]. The increase of CO concentration in lung epithelial cells induces the up-regulation of autophagic marker (LC3B), inhibits the production of ROS in lung epithelial cells and promotes cellular survival [56]. It has been demonstrated that the protective mechanism of CO is compromised by siRNA directed LC3B in A549 lung epithelial cells under hyperoxia. In summary, CO, downstream of HO-1 and upstream of LC3B, is another crucial factor that regulates both apoptosis and autophagy.

P62 and Fap-1

*p62 and Fap-1, emerging central check points of the apoptosis and autophagy*

Another molecule that serves as a regulator for both autophagy and apoptosis pathway is p62/Sequestosome 1. p62 is a receptor that mediates ubiquitinated proteins for autophagic degradation [59]. Particularly, this protein forms a complex with the autophagic marker, LC3B, to regulate the pro-apoptotic factor BID [60]; this regulation, however,

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**Fig. 5. Panel A.** At basal level, the LC3B, Cav-1, and Fas form a heterotrimeric complex that suppresses apoptosis. **Panel B.** Under cigarette smoke extract (CSE), the heterotrimeric dissociates causing apoptosis and autophagy. **Panel C.** Under cigarette smoke extract (CSE) in Cav-1 knockdown, the lack of the heterotrimeric complex induced a greater apoptotic and autophagic activity in epithelial lung cells. **Panel D.** Under cigarette smoke extract (CSE) in LC3B knockdown, the lack of LC3B caused a decrease in DISC formation and apoptosis.
cannot be solely activated by p62 alone but firmly requires LC3B [60]. The active form of pro-apoptotic factor BID, truncated BID (tBID), activates the mitochondrial mediated apoptosis by triggering the release of Cytochrome c [61]. This active form is commonly ubiquitinated [61]. The ubiquitinated tBID binds to p62’s ubiquitinated protein-binding domain for autophagy mediated tBID degradation [62, 63]. The elevation of both tBID protein level and Caspase-3 cleavages after deletion of p62 show the necessity of p62 for cell survival in Beas2B lung epithelial cells [60]. Similarly, under hyperoxic conditions, lung epithelial cells with p62 mutation in ubiquitin-associated domain (UBA) or in LC3-interacting region (LIR) have a significant decrease in survival compared to cells with non-mutant p62 proteins, demonstrating that physical binding of p62/LC3B complex with tBID is crucial for cell survival, as mentioned above [60]. Mechanistically, under hyperoxia exposure, p62 loses its physical interactions with tBID and LC3B, thus decreasing in tBID degradation and causing an accumulation of this pro-apoptotic factor [60].

This apoptosis regulation factor, p62, simultaneously regulates LC3B. p62 has a negative correlation with LC3B; the over-expression of p62 down-regulates LC3B and deletion of p62 causes an over-expression of LC3B [60]. Interestingly, it is also noted that Cav-1 is essential for the physical binding among p62, LC3B, and tBID [60]. This demonstrates another case in which an autophagic factor regulates the apoptotic pathway.

Fas–associated phosphatase 1 (Fap-1), a phosphatase found in non-mitochondrial-involved Fas-induced cell death or Type I cells (BJAB and SKW6.4), has been recently identified as an inhibitor for apoptosis [64, 65]. However, the expression of Fap-1 is not detected in type II cells or mitochondrial-involved Fas-induced cell death (Jurkat and CEM) under both autophagy blocked and non-blocked situations [65]. This phosphatase, Fap-1, dephosphorylates the apoptotic Fas death receptor, decreases the expression of ligand receptor, and ultimately hinders the activation of the apoptotic pathway [64, 65]. In the activation of apoptosis, Fap-1 is found to be degraded by autophagy. During the initiation of apoptosis in type I cells, Fap-1 associates with the autophagic adaptor protein, p62, for autophagic degradation of Fap-1. This pro-autophagic interaction between p62 and Fap-1 is found to be Fas-ligand dependent suggesting further cross-talks between the two cellular death pathways [65].

In the lung, both apoptosis and autophagy are important processes to maintain homeostasis of lung microenvironment and epithelium. Without cellular death or recycling of macromolecules, the cells will act malignantly forming tumors. Interactions between autophagy and apoptosis are crucial for potential cellular adaptation to a new microenvironment blocking them from immediate apoptosis. Without this mechanism, cells are more prone to apoptosis. Autophagy serves as a last layer of cytoprotection against cellular damage. Finding the target for regulation of both apoptosis and autophagy can potentially preserve crucial cells under morbid conditions. The cross-talks between apoptosis and autophagy are not only limited to the lung epithelial cells, but also all other important cells and tissue, as we summarized above. The details on the cross-talks between autophagy and apoptosis in lung epithelial cells require vigorous studies in the near future.

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Conflict of interests

The authors declare no conflict of interest.

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