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Review

Role of Autophagy in Auditory System Development and Survival

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Abstract: Autophagy is a natural catabolic process of the cell that dismantles the useless or dysfunctional components. Autophagy allows the systematic and the lysosomal-mediated deterioration of cellular organelles. During the embryonic development, autophagy plays a critical role by remodeling the tissue and organs of the body, and the deletion of some of the autophagy related genes results in the defective embryonic development. Inner ear is the most sophisticated organ of the body responsible for the sound perception. In mammalian inner ear, autophagy protects the hair cells (HCs) from drug and noise induced damage. In this review, we particularly discuss how autophagy implicates during the auditory system development in mammals and presents its role in age-related hearing loss. Moreover, we discuss the protecting effects of autophagy after noise and drug induced auditory trauma.

Keywords: autophagy; autophagosome; auditory system development; age-related hearing loss; noise and drug induced hearing loss

1. Introduction

The art of hearing relies on peripheral and central auditory system. The acoustic signals in the environment cause the vibration of the tympanic membrane, which enters through the external auditory canal of the auditory periphery system. Then, these signals are conducted and amplified by the ossicular chain of middle ear and transformed into electrical signals by the inner ear HC in the organ of Corti. This is followed by auditory nerve, cochlear nucleus (CN), superior olivary complex (SOC), nucleus of lateral lemniscus (NLL), inferior colliculus (IC), medial geniculate body (MGB), and finally transferred to the auditory cortex (AC) [1,2]. The normal development of the auditory system is important for the normal hearing and understanding the language.

Autophagy is initiated by the formation of double membrane cytosolic vesicles, called the phagophore or isolation membrane [3]. The phagophore matures into a closed double-membrane-bound structure and then fuses with lysosomes, called the autophagosome [4]. Autophagy plays an important role in the survival of living cells, particularly in the stress conditions, such as starvation and oxidative stress [5–7]. Autophagy also participates in regulating many

physiological processes such as cell survival, death, proliferation, and differentiation [7–9]. Autophagy dysfunction has been suggested to induce several pathological changes, such as cancer, inflammation, neurodegenerative diseases, and metabolic disorders [10–12]. In the inner ear, autophagy is a kind of essential catabolic mechanism which is necessary for the embryonic development. The mechanism also responds to otic injury in the adult mouse for autophagic recycling the intracellular components, and eliminates the deleterious molecules and organelles [13]. Inhibition of autophagic pathway provokes the degeneration of HCs, the damage of neurogenesis and the aberrant axonal outgrowth that ultimately leads to hearing loss [14]. Therefore, autophagy has an important role in hearing acuity and maintain inner ear development.

2. Autophagosome Formation and Regulation

The formation of autophagosome is a dynamic process that involves the membrane formation and fusion. In mammalian systems, the autophagosome (omegasomes) is an endoplasmic reticulum (ER) associated structure formed after autophagy induction [15,16]. Following autophagosome initiation, the isolation of double-membrane subsequently expands and surrounds the cytoplasmic components and finally fuse to form the autophagosome [17,18]. After completion of the autophagosome, it reached right destination and the autophagosome membrane will then fuse with lysosomal/vacuolarmembrane [19,20]. Finally, this results in the degradation of autophagosome inner membrane and intra-autophagosomal components [21,22] (Figure 1).

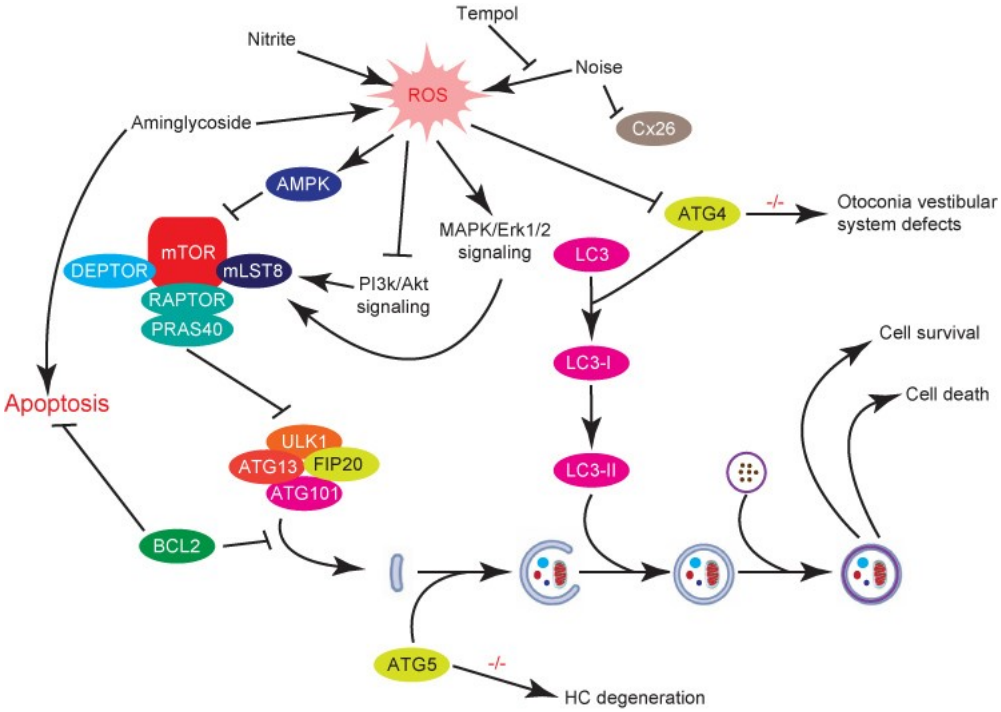

Figure 1. Autophagy signal pathway and the relationship with auditory system.

There are more than 35 autophagy-specific (Atg) genes responsible for autophagosome formation in mammalian orthologs [23,24]. These ATG proteins introduce orchestrated action and are recruited at the initiation, elongation, and closure of autophagosome. Atg1, Atg6, Ambra1 and Vps34 genes all participate in the development of early autophagosome formation [25], while the process of autophagosome membrane maturation is regulated by Atg5-9 and Atg12-14 genes [26]. The elongation of mature autophagosome membrane requires the Atg12-Atg5 and Atg8(LC3)-PE (phosphatidylethanolamine) ubiquitin-like conjugation systems [4,27] and the formation of Atg12-Atg5-Atg16 complex. First, Atg12 is activated by the E1-like enzyme Atg7 and then transferred

to Atg10, and conjugates with Atg5 to form Atg12-Atg5 conjugates [28–30]. Then, this Atg16 binds to Atg5 to form a functionally active complex [31]. Secondly, the process of Atg8/LC3 system begins when Atg8 is cleaved by Atg4 [32]. Atg8 is activated by the same E1-like enzyme Atg7 and then transfered to the E2-like enzyme Atg3 [33]. Finally, the C-terminal glycine of Atg8 conjugates with the PE to form Atg8-PE [34,35]. The membrane-associated Atg8-PE is further cleaved by the Atg4 to release the Atg8 from membranes, which is an essential recycling component for effective autophagy [32,33,36]. In mammals, there are several Atg8-like proteins such as the LC3 and GABARAP subfamilies, which have multiple functions in the elongation and completion of phagophore [37–39]. LC3-I and LC3-II are the ATG4-processed and PE-conjugated form of LC3, respectively [40]. When autophagy is induced in the cell, the synthesis of LC3 becomes substantially increased [36]. In addition, P62 is a key factor in autophagosome formation and autophagy monitoring. In the process of autophagosome formation, p62 interacts with LC3-bound membrane and forms the membrane elongation template [41]. The LIR motifs of p62, which is a special LC3 recognition sequence, can efficiently bind to the membrane and shuttles ubiquitinated proteins to the autophagosome for degradation. p62 can achieve self-oligomerization from the PB1 domain. The mutation of PB1 can lead to the deficiency of self-oligomerization. Autophagosome cannot be formed and the ability of autophagic degradation weakens in that circumstance, indicating that p62 assemblies and self-oligomerization are necessary for the formation of autophagosome [42,43]. The p62 protein is not only a receptor for ubiquitinated proteins, but also selectively binds to ubiquitinated proteins and sends to the autophagosome for further degradation which can be served as a marker to study autophagic flux. p62 accumulates when autophagy is inhibited, and p62 decreases when autophagy is activated [44].

The whole activity of the autophagy machinery is regulated by the cAMP-dependent protein kinase A (PKA) and the TOR pathways [45]. Inhibition of mTOR complex 1 (mTORC1) by AMPK induce autophagy and activates the UNC51-like kinase 1 (ULK1; also known as ATG1) complex [46]. In addition, there are several other factors involved in the regulation of autophagy including Bcl-2, SIRT1, calcium [47], reactive oxygen species (ROS) [48,49], FOXO3 [50], BNIP3 [51], p19 ARF [52], DRAM [53], calpain [54], TRAIL [55], FADD [56], and myo-inositol-1,4,5-triphosphate (IP3) [57,58]. The transcription factor FoxO3a is activated by the SIRT1 that promotes the expression of several autophagy-related genes including LC3, Atg12, Bnip3, and Rab7 [50,59]. The anti-apoptotic proteins BCL-2 and BCL-XL inhibit autophagy through binding to the beclin1 (BECN1).

3. The Role of Autophagy in Embryogenesis and Development

Autophagy selectively responds to environmental and hormonal signals during embryogenesis and cell differentiation. Several studies have reported ascertaining role of autophagy in mammalian development and diseases using global and tissue specific knockout mice models. Deletion of some autophagy-related (Atg) genes leads to embryonic lethality during mid-embryonic development. Atg gene knockout mice survive the postnatal period, however, display some developmental abnormalities [60]. In mammals, autophagy plays an important role in the elimination of paternal mitochondria post-fertilization after oocyte fertilization [61–63]. In addition, autophagy also regulates different cells (such as erythrocytes, lymphocytes, and adipocytes) differentiation by remodeling the cell cytoplasm [60,64]. The nutrients are restricted in the stage of embryos development [61]. The development of embryos is halted before the blastocyst phase when the mechanism of autophagy in oocytes is deficient [61,65]. Embryonic stem cells fail to form expanded cystic embryoid bodies in Beclin-1-knockout mice [66]. Autophagy is also important for the neuronal development. The ablation of Atg7 causes deficits in the neuronal cells motor function, abnormal swellings and dystrophy of Purkinje cell axon terminals in the deep cerebellar nuclei [67]. The inactivation of Atg1 plays an important role in fiber formation and cerebellar development and this impairs axon outgrowth and differentiation of neurons in immature granular cells [68]. Autophagy also co-regulates the embryogenesis by using some developmental pathways such as Shh, TGFβ, Wnt and FGF, which are important in cell differentiation and cell proliferation [69–72].

4. The Role of Autophagy in Auditory System Development

Hearing loss is the most common sensory disorder in humans, caused by the different etiologies such as congenital morphogenetic defects, aging, exposure to intense noise, ototoxic medications, and genetic disorders [73,74]. The inner ear supports and functionally enables the auditory function by transmitting signals from the sensory epithelia to the brain by using hair cells, adjacent supporting cells, and neurons [75].

Autophagy participates in differentiation and specification of cell fate through energy supplies and cell contents degradation [14,76,77]. Deletion of some autophagy-related genes leads to embryonic lethality or developmental abnormalities [77]. Autophagy has a key role in shaping the otic epithelium and facilitating the process of neurogenesis. During the early inner ear development, complementary levels of autophagy affect the removal of dead cells, and promote the differentiation of otic progenitor and axonal outgrowth of neuron [14]. Inhibition of autophagy in otocyst results in the misregulation of the cell cycle and impaired neurogenesis and poor axonal outgrowth. The transcription inhibition of the LC3B or class III phosphatidylinositol 3-kinase causes the aberrant otic vesicles (OVs) morphology [14]. Atg4b-deficient mice have shown the balance disorder, otoconia, and vestibular system defects. The autophagic activity is necessary for the otoconial biogenesis and it affects the secretion and assembly of otoconial matrix [78]. Autophagy-related genes (ATG) Beclin1, Atg4g and Atg5 are expressed from the late embryonic development period until adulthood in the mouse cochlea, vestibular system, and brainstem cochlear nuclei [79]. Auditory HCs are the core component of the cochlea which are responsible for the auditory functioning and found on the end organ of the inner ear. These HCs are not spontaneously regenerate in the adult mouse cochlea. The Atg5-deficient mice exhibit HC degeneration and congenital hearing loss due to the accumulation of polyubiquitinated protein in the HCs [74]. Therefore, autophagy is important in maintaining the morphology and the function of auditory HCs.

5. The Role of Autophagy in Age-Related Hearing Loss

Ageing is an inevitable physiological process initiated due to the accumulation of damaged molecules and organelles in cells which leads to a progressive organism decline and rises in vulnerability to diseases [80–82]. Age-related hearing loss (ARHL) is a prevalent sensory disorder in elderly individuals and is characterized by a progressive inevitable hearing and speech discrimination decline [83,84]. The accumulation of waste products and various stress molecules in cells are the serious problems in the cell cleaning systems. During aging, the efficiency of autophagic degradation reduces and intracellular ROS accumulates in postmitotic cells. The ROS accumulation caused by the imbalance between the ROS production and the ability of the antioxidants is a fundamental reason in age-related diseases such as ARHL [85,86]. Autophagy is considered as a cytoprotective mechanism against various stress molecules and it helps to remove the harmful constituents in aged or damaged organelles [87]. It is evident that the increment autophagy could possibly extend the lifespan [88]. The process of aging in cell begins due to the oxidative stress and DNA damage, which regulates mTOR signaling and NF-κB signaling that affects autophagosome formation [49,89,90]. The mTOR is a key negative regulator of autophagy and its activity is regulated by multiple signaling pathways such as PI3K-Akt and AMPK. Previous studies have found that excessive ROS can activate autophagy by inhibiting PI3K-Akt-mTOR [91]. The ROS produced during sevoflurane exposure can induce autophagy by activating AMPK and inhibiting mTOR signaling pathway [91]. In the process of autophagosome formation, ROS regulates autophagy by inhibiting the activity of Atg4. ROS can deactivate Atg4 and cause accumulation of LC3-II, resulting in an increase in autophagosomes [49]. In addition, ROS regulates autophagy through the MAPK signaling pathway which plays an important role in cell proliferation, differentiation, stress adaptation and apoptosis, including JNK and ERK. MAPK can regulate autophagy-related gene expression and affect autophagy by regulating the activity of transcription factors such as AP-1, FoxO, and NF-κB. Many exogenous substances such as ROS can activate autophagy through MAPK [92]. Experiments showed that

ROS can induce autophagy in mouse mesenchymal stem cells cultured in vitro through the JNK signaling pathway. Arsenite can induce autophagy through ERK pathway, which is activated by ROS [93]. Several studies have demonstrated that the reduction of mTOR signaling increases lifespan. In the cochlear stria vascularis, autophagy is considered to play a pro-survival function which is modulated by the PARP-1 in oxidative stress-induced stria marginal cells death [83]. In senescence accelerated prone mice (SAMP), the oxidative stress, chronic inflammation and mt-DNA mutations are causal factors triggering premature ARHL [94]. The oxidative stress and chronic inflammation induced the damaged mitochondria and aberrant proteins accumulation which can be removed by autophagy upregulation [95,96]. When aberrant autophagy occurs, the pro-survival ability weakens and the misfolding and nonfunctional organelles and proteins can trigger cell death pathways such as apoptotic and autophagic [97]. In the study of spiral neurons, autophagy not only has a protective effect in young cochleae but also has an apoptotic effect in old cochleae when it is overactivated [94]. The change of expression of intrinsic genes is another endogenous regulation of autophagy in ARHL. Some research found that the changes in the expression of miR-34a can affect cochlear cell apoptosis in AHL via modulating autophagy [98]. The increased miR-34a when autophagy impairment can induce cochlear hair cell death and AHL. In contrast, the decreased miR-34a when treated with ursodeoxycholic acid (UDCA), which is an effective miR-34a inhibitor, can attenuate cell death [98]. miR-34a over-expression inhibits the expression of ATG9A which is related to autophagosome-lysosome fusion. Thus, autophagy has a dual role in life and death at different situations, for example, autophagy plays a protective role when the level of oxidative stress is low in the young cochleae and a pro-apoptotic role when the level of oxidative stress is excessive in the old cochleae. The level of autophagy in the ARHL is not only affected by ROS but also regulated by multiple intrinsic genes. To address the relationship between the underlying genes expression changes and autophagy, utilizing a combination of autophagy inducers and lysosome biogenesis enhancers is a pretty strategy for fighting against the neurodegenerative diseases such as ARHL.

6. The Role of Autophagy in Noise and Drug Induced Hearing Loss

Although recent studies reported that the mouse cochleae have very limited HC regeneration ability in the neonates, this limited spontaneous HC regeneration is not able to recover the hearing ability once HCs are damaged, and adult mice completely lose this HC regeneration ability as they aged [99–102]. Use of ototoxic drugs (such as chemotherapeutics, aminoglycosides, and loop diuretics) and noise induced hair cell (HC) damage are the main causes of sensorineural hearing loss [103,104]. ROS overproduction and reactive nitrogen species (RNS) are the common pathologic characteristics emerging in the cochlear tissues after noise exposure and ototoxic drug treatment [105]. Cisplatin is often used as a treatment for tumors, but its use is limited by its ototoxic effects which induce ROS increase, mitochondrial depolarization reduction and mitochondrial damage, resulting in hair cells apoptosis [106]. Cisplatin can trigger autophagy and activate multiple autophagy-related factors such as Akt and c-Jun. In general, aminoglycoside-induced dysfunction of mitochondria can activate mitophagy, but some research found that gentamicin and neomycin cannot activate the mitophagic machinery in HEI-OC1 cells and mice's organs of Corti (OC) [107,108]. In addition, when treated with carbonyl cyanide m-chlorophenyl hydrazone (CCCP), which can generate mitochondrial depolarization, mitophagy was activated in HEI-OC1 cells and OC. These results indicate that mitophagy is an independent mechanism and not a universal response to mitochondrial damage after aminoglycosides injury. The rapamycin has an otoprotective effect. It attenuates the cisplatin-induced ototoxicity through inducing autophagy and attenuating oxidative damage [109]. Tempol, a stable radical scavenger, is another drug which exerts an inhibitory role against cisplatin-induced ototoxicity. Mitochondrial damage and increase in superoxide anions caused by cisplatin-induced oxidative stress can be inhibited by Tempol [110]. Thus, autophagy plays an important role in the process of ototoxic drugs-induced hair cell apoptosis and survival. Inhibition or enhancement of autophagy causes changes in the level of reactive oxygen species and apoptosis in hair cells. Another main

cause inducing hearing loss is noise, which induces ROS and NO accumulation and connexin26 down-regulation that results in the spiral ligament damage. Autophagy may play a dual role in cell survival at the early stage and cell death at the late stage of autophagy after exposure to stress [111]. The lower levels of oxidative stress incurred by TTS-noise exposure or aminoglycosides induces autophagy, which inhibits apoptosis and protects the HCs by suppressing ROS accumulation [104,108]. In contrast, the excessive accumulation of giant non-functional mitochondria and ROS induced by higher levels of oxidative stress might trigger autophagic stress, which in turn could induce cell death [108]. Previous research found Tempol can prevent hearing loss through inhibits noise-induced JNK pathway up-regulation and connexin26 down-regulation. Thus, the maintenance of normal mitochondrial function and ROS level by autophagy is important for the survival of HCs after noise or ototoxic drug-induced injury. In noise and drug induced hearing loss, the abnormal autophagy caused by the increase of ROS is an important cause, but the autophagy is not only activated by ROS, but also by the regulation of various related genes (Figure 1). It is equally important to find which gene expression changes during this process will lead to abnormal autophagy. Combination therapy of autophagy-related drugs and regulation of target genes will provide important assistance in improving noise and drug induced hearing loss.

7. Conclusions

Autophagy is a major mechanism involved in the clearance of cells from oxidative stress or otherwise damaged and worn-out macromolecules and organelles. In the inner ear, autophagy plays an important role in maintaining the morphology and the function of the auditory system. Moderate ROS levels promote autophagy to recycle damaged cellular constituents and maintain cellular homeostasis, while the induction of autophagy inhibits apoptosis and protects the HCs by suppressing ROS accumulation in aminoglycoside injury, aging and noise exposure. Autophagy considerably regulates numerous aspects of mammals through embryogenesis and development, such as neuronal system, immune system, etc. Autophagy resists various stress molecules such as ROS and helps to deplete the harmful constituents in the aged. These processes are conducted mainly by activating AMPK and inhibiting mTOR signaling pathway. Other intrinsic genes such as miR-34a, ATG9A, and PARP-1 also regulate autophagy to play a role in ARHL. Noise exposure and ototoxic drug treatment, including Cisplatin and CCCP, cause eternal hearing loss via producing ROS and RNS, which are opposite side of autophagy. Autophagy maintains normal mitochondrial function and ROS level for HC survival after aforementioned injury. Drugs such as rapamycin and Tempol play an otoprotective effect by suppress ROS (Figure 1). Abnormal autophagy caused by excessive ROS is also a concern. Corresponding treatment for pro-autophagy and anti-ROS should be focused on more in the future.

Conflicts of Interest: The authors declare no conflict of interest.

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