An Overview of the Perspective of Cellular Autophagy: Mechanism, Regulation, and the Role of Autophagy Dysregulation in the Pathogenesis of Diseases

Yasser M. Alharbi[1], Abdulhadi I. Bima[1], Ayman Z. Elsamanoudy[1,2]

[1]Department of Clinical Biochemistry, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia, [2]Medical Biochemistry and Molecular Biology, Faculty of Medicine, Mansoura University, Mansoura, Egypt

Abstract

Autophagy is a cellular process that eliminates unnecessary cytoplasmic materials, such as long-age proteins, destroyed organelles, and foreign microorganisms. Macroautophagy (MaA), chaperone-mediated autophagy, and microautophagy are the three main types of autophagy. It is regulated by the integration of signaling from the AMPK and mTOR-ULK1 pathways. Autophagy plays a physiological role in health, and its dysregulation could be a pathophysiologic mechanism in different disease conditions. In the current study, we reviewed papers of Google Scholar database, PubMed, PubMed Central, Cochrane Database of Systematic Reviews, MEDLINE, and MedlinePlus with no time limitation and a recent World Health Organization report. In the current review, it could be concluded that autophagy plays many physiological functions, including immune system modulation, and regulates different cellular processes such as metabolism, protein synthesis, and cellular transportation. Dysregulation of autophagy is implicated in tumorigenesis, aging, age-related neurodegeneration, and endothelial dysfunctions. Autophagy dysregulation is also implicated in the newly discovered CoV-COVID-19 pathogenesis.

Keywords: Autophagy, COVID-19, dysregulation, endothelial dysfunctions

Introduction

Autophagy process is mediated by formation of a cellular organelle called an autophagosome. This autophagosome is transported to the lysosome of the cell to get rid of any unwanted cellular debris. A group of protein molecules called autophagy-related proteins (ATGs) are responsible for mediating this autophagy process.[1]

Accumulating evidence discussed the magnitude and value of autophagy in various diseases, including malignancy, neurodegenerative disorders, metabolic disturbance, immunity-related diseases, and infectious diseases. Moreover, the involvement of autophagy dysregulation in viral infection had been widely investigated and considered.[2]

Coronavirus disease 2019 (COVID-19) is a newly discovered disease that is caused by RNA virus belonging to Coronaviridae family. It is described as the pathologic agent of a severe acute respiratory syndrome that was initiated in December 2019 in Wuhan, China, by the World Health Organization (WHO).[3]

The significance of autophagy in the pathogenesis of COVID-19 and/or its potential therapeutic values is unclear and under debate.

The objective of this review article is to focus on autophagy mechanism, regulation, and its possible embroilment in the pathogenesis of diseases. Moreover, it aims to discuss also the potential involvement in COVID-19 pathogenesis.

Address for correspondence: Prof. Ayman Z. Elsamanoudy, Department of Clinical Biochemistry, Faculty of Medicine, King Abdulaziz University, Jeddah 21465, Saudi Arabia.
E-mail: ayman.elsamanoudy@gmail.com

Access this article online

Quick Response Code:

Website:
http://www.jmau.org/

DOI:
10.4103/JMAU.JMAU_33_20

METHODS

In this study, we reviewed published articles of PubMed, PubMed Central, Cochrane Database of Systematic Reviews, MEDLINE, and MedlinePlus as well as Google Scholar database and WHO report. There is no time limitation of the articles that are used in this review

Review

Definition of autophagy

"Autophagy" was first discovered and described by the Nobel Prize winner (1974), "Dr. Christian de Duve," a Belgian Biochemist.[4] "Autophagy" is a scientific term that is derived from the Greek word "*autóphagos*-eating of self."[5]

It is a cellular process that eliminates unnecessary cytosolic materials (long-age proteins, destroyed organelles, and foreign microorganisms, by the degradation of lysosomes). All these materials or contents are sequestrated into autophagic double-walled vesicles (autophagosomes), which then lastly fuse with the lysosome (autophagolysosome).[6]

Among other lysosomal pathways of degradation, "Autophagy" is a unique process as it is considered the only process involving autophagosomes formation for the delivery of cargo to the lysosomes.[7] Apparently, autophagy is a critical and tightly regulated catabolic mechanism for the maintenance of cell survival during development, hunger, and cellular differentiation. It also plays basic biological cellular functions. There are various stresses that induce autophagy at a rapid rate (as opposed to the basal rate under normal conditions) such as starvation, aggregation of malformed proteins, damaged organelles plus inflammations.[8]

Types of autophagy

Autophagy may be classified according to the substrates undergo degradation into: (1) endoplasmic reticulum (ER-phagy), (2) mitochondria (mitophagy), (3) ribosomes (ribophagy), (4) lysosomes (lysophagy), and (5) peroxisomes (pexophagy).[9]

Three main pathways describe "bulk autophagy," namely "Macroautophagy" (MaA), chaperone-mediated autophagy (CMA), and microautophagy. Other types of autophagy that are more specific are also documented such as the selective breakdown of mitochondria (mitophagy), breakdown of lipids (lipophagy), and selective elimination of foreign pathogens (xenophagy).[10]

In CMA, specific substrates with KFERQ-like motif are selected via heat shock; HSPA8/HSC70 protein targets these substrates to the lysosomal surface and helps in the binding of those substrates to lysosome-associated protein type 2A (LAMP-2A).[11] This LAMP-2A undergoes multimerization as a result of binding of the substrates, then the substrates are translocated into the lysosome, and finally, subsequent breakdown with the help of lysosomal proteases.[12]

In microautophagy, the lysosomal membrane undergoes invagination to form a vesicle in which the substrates are trapped and later degraded by lysosomal protease as they are pinched off from lysosomal membrane.[13] The end products of these autophagy degradation systems are reused in various cellular metabolic synthetic processes as protein, lipid, and adenosine triphosphate (ATP) production.[7]

Mechanisms of autophagy

Induction of autophagy

Autophagy is a strictly controlled mechanism that can either lead to large-scale breakdown of content within the cells due to inflated energy requirements or selective removal of different cytoplasmic products of cellular damage and/or stress.[5] Low-level autophagy plays a role as cytosolic quality control and adaptation mechanism of cellular stress under physiological conditions, thus ensure cellular homeostasis. Autophagy is triggered to facilitate survival under adverse circumstances in response to stress (nutrient deficiency, hypoxia, and oxidative stress) and extracellular signals. These trigger autophagy to induce macromolecule recycling or to degrade harmful organelle-producing reactive oxygen species (ROS).[7,14]

Starvation is a classical induced of autophagy mechanism, while in nutrient-rich conditions, mammalian target of rapamycin (mTOR) inhibits this mechanism.[15] Transcription factor EB (TFEB), the principal controller of autophagy, controls the gene expression required for the biogenesis of autophagosomes and lysosomes.[16] Extensive yeast studies have established ATG proteins as the principal components that facilitate autophagy activation and maintenance. The bulk of these proteins is contained in systems of mammals.[17]

Autophagy pathway

The pathway remains inactive under nutrient-rich conditions, but due to mTOR-dependent phosphorylation or stress, MP-activated protein kinase (AMPK) activates, promoting the initiation of autophagy, via mTOR downregulation and concomitant unc-51-like kinase 1 (ULK1) initiation. When the ULK1 component is operational, it is transported to the preautophagosomal membrane structures to initiate phagophore creation. ULK1- and AMPK-dependent Beclin1 phosphorylation disrupts its association with anti-autophagic components (such as Bcl-2) and increases the uptake of complex sorting vacuolar protein 34 (VPS34). Subsequently, the active VPS34 complex functions as a phosphoinositide 3-kinase-class III (PI3K) to produce phosphatidylinositol 3-phosphate (PI3P). The resulted PI3P interacts with WD-repeat protein interacting with phosphoinositides (WIPI) proteins, promoting the uptake of ATG proteins, necessary for the nucleation of phagophore. The important step for the autophagosomes formation is the coupling of microtubule-associated protein light chain 3 [LC3]/ATG8 to phosphatidylethanolamines lipids, a reaction involving the coordinated work of ATG7, ATG3, and ATG5-12-16. On the other hand, ATG9 helps in autophagosome formation since it supplies the growing phagophore with lipids. Specific cytoplasmic adapters (as proteinclumps or degraded mitochondria) in the phagophore during expansion, through direct contact with LC3II are also involved. A scission event comes to regulate the phagophore to wind up and produce a bilayer autophagosome. The newly developed autophagosomes

join to connect lysosomes. Multiple proteins, including soluble N-ethylmaleimide-sensitive factor plus homotypic fusion and protein sorting, aid autophagosome–lysosome attachment. The outer autophagosomal membrane fuses with the inner lysosomal membrane forming an autolysosome. Effective acidification (mediated by v-ATPase) initiates hydrolysis of the lysosomes, then gradually breaks down the sequestered cytosolic material.[9,18-20]

Autophagy regulation

The integration of the AMPK and mTOR-ULK1 signaling pathways activates autophagy in endothelial cells (ECs). AMPK reveals any lowered cellular ATP levels and consequently activate autophagy.[21] The mechanism is mediated by that AMPK phosphorylates tuberous sclerosis 2 protein; this leads to inhibition of mTOR which activates autophagy in a similar mechanism to that occurs during starvation. Moreover, CaMKK-β induces AMPK activation that inhibits mTOR and upregulates ULK1. This leads to autophagy activation. Hence, a dynamic interplay between AMPK, intracellular calcium, mTOR, and ULK-1 in ECs is the mechanism involved in the activation of autophagy.[5]

Autophagosome

Autophagosome is the cytosolic hallmark and the key event of autophagy. The size and number of autophagosome may be regulated by a member of the ATG8/LC3 protein family.[22]

Biogenesis of autophagosome is mediated by coincided activities of autophagous proteins, as ULK1 and PIK3C3/ VPS34 act as upstream and central regulators in autophagosome biogenesis. Furthermore, for autophagosome formation, two ubiquitin-like conjugate systems are needed for phagophore membrane fusion. These ubiquitinated protein groups are ATG12-ATG5-ATG16 L1, LC3-II, and ATG9.[4]

"Autophagy Adaptors for Selective Autophagy"

According to the current evidence, selective autophagy has been discovered responsible for the cellular quality control mechanism that functions against aggregated or malformed protein inclusions (aggrephagy). Moreover, it is a defense mechanism against accumulated defective cellular organelles such as mitochondria (mitophagy), ER (reticulophagy), peroxisomes (pexophagy), ribosomes (ribophagy), and infectious pathogens (xenophagy).[23,24]

Mitophagy is of great importance among the different types of selective autophagy because mitochondria have a great role in maintenance as well as destruction of a cell through its role in regulating apoptosis. Hence, dysfunctional mitochondria are incorporated in the pathogenesis of human diseases.[25,26] Mechanical insight into selective autophagy was provided by the recognition of several autophagic adapters molecules such as p62/SQSTM1, neighbor of the BRCA1 gene, optineurin, nuclear domain 10 protein 52, and TAX1 binding protein 1. Adding to these adapters of selective autophagy, it has been realized that the development of the destructive products, in particular ubiquitination, makes sure the identification of the substrate and the specificity of autophagy adapters.[4,27]

Table 1: Examples of genes that are involved in autophagy mechanism

Gene/affected protein	Link to autophagy
Genes that regulate autophagy	
AKT	Gain-of-function mutations/amplifications in the oncogenes PI3K and Akt
PI3K	Loss-of-function mutations in the tumor suppressor gene PTEN
PTEN	These genes have an inhibitory effect on autophagy
TSC1	TSC
TSC2	Mutations in TSC1 or TSC2 abolish their inhibition of mTOR, leading to decreased autophagy
LKB1/STK11	Somatic mutations in nonsmallcell lung cancer. LKB1 activates AMPK and stimulates autophagy
p53	Mutations in p53 mutations are found in >50% of all human tumors
	p53 may activate autophagy after genotoxic stress
Bcl-2	Amplification of Bcl-2 may inhibit autophagy by targeting Beclin 1
IRGM1	IRGM1 is an immunity-related GTPase that stimulates autophagy
Gene products that are required for autophagy	
Atg16L1	Atg16L1 is involved in autophagy-dependent immune regulation or bacterial clearance
Beclin 1	Beclin 1 is involved in cell growth control associated with reduced autophagy
UVRAG	UVRAG has an effect nearly similar to Beclin 1 deletion
Genes products involved in autophagosomal sequestration, movement, or maturation	
P62/SQSTM1	P62/SQSTM1 is involved in the regulation of ubiquitin-binding site of protein
	So, P62/SQSTM1 mutation leads to impaired autophagy of ubiquitinated proteins
Dynactin subunit p150[glued]	It is involved in abnormal movement of autophagosomes
CLN3	CLN3 mutation could be linked to reduced autophagosome/lysosome fusion
LAMP-2	LAMP-2 mutation could be linked to reduced autophagosome/lysosome fusion
	It is linked to Familial X-linked cardiomyopathy

TSC: Tuberous sclerosis complex, mTOR: Mammalian target of rapamycin, LAMP-2: Lysosome-associated protein type 2

Genetic and molecular aspects of autophagy

Regulation of autophagy at the transcriptional level

It is reported that TFEB is known as one of the main autophagy controllers of autophagy at the transcriptional level.

TFEB combines certain autophagy and lysosomal genes to the Coordinated Lysosomal Expression and Regulation component. This is important to initiate their expression which enhances biogenesis of both autophagosome and lysosome. It also helps in autophagosome–lysosome fusion.[28] TFEB transcription activity is closely regulated by phosphorylation. ERK2, AKT/PKB, and mTORC1 mitochondria are known to inhibit TFEB by sequestration in the cytoplasm under nutrient-rich conditions.[29] In comparison, under conditions such as starvation, the signal blunt mTORC1, inhibitory phosphorylation on TFEB decreases and TFEB transports to the nucleus.[4]

Regulation of autophagy at the level of posttranslational modifications of autophagy proteins

The mechanism of "Post-Translational Modifications on Autophagy Proteins and the Regulation of Autophagy" is described by Chun and Kim[4] as follows: there are two key autophagy-initiating kinase complexes, the ULK1 and PIK3C3/VPS34 complex are involved in this mechanism.

The ULK1 complex is controlled by phosphorylation. This phosphorylation is catalyzed by AMPK. It induces multiple phosphorylations of the catalytic subunit ULK1. It is recognized that it is inhibited by phosphorylation of mTORC and ATG13. On the other hand, the ULK1 complex is activated by conjugation with ubiquitin, while it is negatively regulated by degradation based on ubiquitisation. In response to depletion of the growth factor, the activated GSK3-TIP60 acetyltransferase axis increases the acetylation of ULK1, which results in induction of autophagy. ULK1 can comprise of a negative feedback loop for its upstream regulators, AMPK and mTORC1, by phosphorylizing the Raptor protein subunit mTORC1 and all complex subunits of AMPK. Regarding PIK3C3/VPS34 complex, phosphoregulation of this complex is detected in the catalytic subunit VPS34 lipid kinase, BECN1, ATG14 L/Barkor (mTORC1 for inhibition), and UV Radiation Resistance-Associated Gene Protein (UVRAG). Ubiquitination of VPS34, BECN1, and ATG14L/Barkor is also of importance in the regulation of autophagy.[4] Examples of genes that are involved in autophagy mechanism are listed in table (1) by Levine and Kroemer.[30]

Physiological role of autophagy

The autophagy plays a wide variety of physiological functions. It works decisively in various cellular systems, including immune systems, often in a coordinated, interconnected manner. It is involved in a normal cellular physiological processes such as metabolism and cellular transportation.[5,31]

Autophagy's function in adaptive and innate immunity involves the removal of microorganisms, regulation of inflammation, immune mediator production, and antigen presentation.[12]

Since autophagy is responsible for the selective breakdown of unnecessary and deleterious cell survival components, it is considered to be a major contributor to homeostasis within the cell.[9]

Autophagy can directly eradicate foreign microorganisms by the mechanism of xenophagy, induction of MAP1LC3/LC3-associated phagocytosis, and sequestosome-like receptors.[32] Autophagy controls inflammation, in particular by affecting TLR signals and bypassing inflammatory activation. Autophagy also regulates inflammation through regulatory interactions with innate immune signaling pathways by removing endogenous inflammatory agonists and by impacting on the secretion of immune mediators, such as cathepsin K, lysozyme, interleukin (IL)-6, IL-8, and molecular patterns associated with damage. Autophagy plays an important role in the recruitment, activation of maturation, and polarization of cell repertoire T. This is also essential to the survival and functioning of B1 cells and plasma cells.[12,33]

Autophagy participates in the presentation of MHCII antigen and could also affects the MHCI presentation.[12] Neurons are often protected from proteotoxicity because of autophagy which is one of the principal proteostatic systems and its involvement in the removal of clumped proteins and destructed cellular organelles.[9]

It is documented that autophagy plays a role in lipid metabolism by a mechanism that involves selective breakdown of lipid autophagy and lipolysis of triglycerides.[34] A hepatic ATG7 knockout mouse has been reported to have shown dysfunctions in the degradation of lipids. Contrary to the ability to break down lipids, however, autophagy also includes the development of white adipose tissue.[4,35]

Effect of dysregulation of cellular autophagy

Autophagy and carcinogenesis

Autophagy is involved in tumorigenesis. In cancer, autophagy has a dual role. In a premalignant phase of tumorigenesis, it acts as a suppressor of oncogenesis by maintaining cellular homeostasis and degradation of cytotoxic material. It also inhibits oncogenes.[36] In the malignant stage and the malignant tumor is formed, autophagy maintains cancer cells survival, especially in the hypoxic area of the tumor, this could lead to more growth of the tumor even becomes more resistant to chemotherapy and more vulnerable to metastasis.[7,37]

However, a recent metabolome study has shown that lack of oxygen increases protein and lipid catabolic products to provide a basal level of mitochondrial ATP production.[38] In this study, it is reported that the inhibition of autophagy decreases the amount of intracellular ATP and consequently induces cell death.[39,40]

It is also reported that many advanced tumors developed what is called autophagic addiction. This could be explained by the need of these tumors to maintain their energy balance by recycling intracellular components for the purpose of energy production.[7]

Kang *et al.*[41] have reported amelioration of many various cancers with several ATG genes (such as ATG2, ATG5, ATG9, ATG12, and UVRAG).[41] For examples, BECN1 is detected in malignancy of the breast, ovaries, and prostate;[4] PIK3C3/VPS34 complex decreased in gastric and prostate cancers.[4,42]

A direct association between tumor progression and the protein level of p62/SQSTM1 of the autophagy adaptor has been reported *in vivo* study.[43] The accumulation of p62/SQSTM1 is induced by the suppression of autophagy which has been observed to enhance ER stress and DNA damage and to suppress kappa B nuclear factor and the erythroid 2-related antioxidant nuclear factor 2 pathways in many carcinogenic cells.[44] Therefore, autophagy dysregulation is most probably responsible for the production of ROS and its associated rise in damaged DNA damage in the form of double strand breaks.[4]

"Autophagy and Ageing"

It is proved that mitochondrial DNA mutations and oxidative stress are considered as causative agents of both aging and neurodegeneration.[9] Aging is associated with many phenonomna as diminished nutrient sensitivity, cellular organelle dysfunction, enhanced cellular aging events, and stem cells functions defect. Autophagy is the principal cellular mechanism that preserves proteostasis and mitochondrial dysfunction. It is suggested that autophagy dysfunction may be at least partly due to aging and vice versa.[45]

This hypothesis could be supported by several evidences that includes the detected downregulation of Atg5, Atg7, and Beclin 1 autophagy genes, an increased mTOR activity, followed by a decrease in ATG protein levels in aged brains.[9,46] Moreover, human skin fibroblasts of healthy people show reduced mitophagy activty and mitochondrial biogenesis in an age-related manner.[9,47]

Several studies consider a close correlation between aging and downregulation or autophagic activity deregulation.[48] Ironically, autophagy is controlled primarily by several metabolic pathways and food intake, which have been shown to affect diseases linked to aging. Autophagy is modulated by insulin/insulin-like growth factor-1 (IGF-1) signaling, mTOR pathway, and dietary deprivation and starvation.[49] Importantly, genetic or pharmacological treatments that prolong the lifespan by reducing the insulin/IGF-1 signalling, mTOR inhibition, or histone deacetylation and dietary restriction often increase autophagy, although some studies indicate that their antiaging effects involve autophagy.[9,50]

"Autophagy and Age-Related Neurodegeneration"

Dysregulation of autophagy was found in Alzheimer's disease, as autophagosome-like structures accumulate in Alzheimer's disease animal model.[51] Following Alzheimer's disease, Parkinson's disease is the second most common neurodegenerative condition.[52] Indeed, a number of reports indicate a blockade of Parkinson's Disease (PD) autophagy. Postmortem examination of brain samples of patients with Parkinson's disease reveals deposition of autophagosomes and loss of lysosomal markers in dopaminergic nerve cells.[53]

Endothelial dysfunctions and autophagy

Higher vertebrate ECs create the lining of all sub-vascular compartments that is responsible for supply nutrients and oxygen to all tissues, thus maintaining integrity and homeostasis of the tissue/organism.[7] Vascular homeostasis is highly dependent on the proper actions of ECs, and thus, no unexpected changes in the biological function of the main EC induced by pathological injury or aging are related to atherosclerosis, neurodegeneration disorders, and cancer.[54]

Nowadays, vascular autophagy is implicated in the development of many age-related vascular diseases. It is evidenced by that autophagy is important for vascular development during intrauterine embryogenesis, and later, the expression of the autophagy proteins (ATG7, ATG8, and Beclin1 [BECN1]) in the angiogenic plexus vessels associated with proper EC–EC junctions, hence its role in protection against hemorrhagic states.[55]

ECs are often found in healthy organisms in a dormant steady state but still have the ability to respond to any changes or stimuli in the surrounding microenvironment which mostly occurs in the form of angiogenesis in response to the reduced oxygen or nutrients supply, then ECs return back quiescent on the restoration of oxygen and/or nutrients supply to the physiological state.[56]

As a part of the age-related physiological changes, there is an increased risk of cardiovascular disease, and the autophagy mechanism is reported to be compromised in the aged ECs.[7,57] In addition to lowered levels of pro-autophagic proteins, BECN1 and LC3 are found in aged mice ECs model compared to young animals controls.[7,58] Another evidence of autophagy dysfunction and its relation to endothelial dysfunction is that reported by Vion *et al*.[59] They reported that genetic inactivation of the gene of EC autophagy in an atherosclerotic animal model is associated with an increase atherosclerotic plaque formation even in areas that are usually resistant to plaque development.[59] This proved the atheroprotection role of autophagy under physiological conditions.[7,60] Moreover, it is documented in animal study that dyslipidemia, especially high level of oxidized LDL-C exposure, induces autophagy in ECs, as a defense mechanism against possible atherogenesis, this indicates that autophagy-mediated lipid (lipophagy) homeostasis promotes normal vascular integrity and function.[7] Imapired autophagy mechanism is involved in many endothelial dysfunction-related diseases as diabetic nephropathy.[61,62] Impairment of autophagy is involved in the pathogenesis of diabetic nephropathy by the initiation of the rapamycin (mTOR) mechanistic target pathway. mTOR is composed of two distinct signaling complexes, mTOR Complex 1 (m-TORC1) and mTORC2, which control autophagy. In general, ULK1 phosphorylation inhibits autophagy by mTORC1. Nutrient deficiency primarily induces autophagy by inhibiting mTORC1.[62,63]

In patients with Danon diseases, the accumulation of glycogen granules in the cardiac and skeletal muscles causes

cardiomyopathy and proximal myopathy.[4] It is attributed mainly to defective autophagy.[64]

Autophagy and COVID-19

Coronaviruses (CoVs) are single-stranded RNA enveloped viruses (26–32 kilobases in size).[65] They belong to the Coronaviridae family of viruses in the order *Nidovirales*. There are three groups of CoVs: α-CoVs, β-CoVs, and γ-CoVs.[66] Severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) are subtypes of two of the β-CoVs. They are the pathogenic causes of severe acute respiratory disease epidemic in China in 2002 and the Middle East in 2012.[67] Recently, in 2019, coronavirus 2 (SARS-Cov-2) started in China. It is named SARS-Cov-2. This SARS-CoV-2 showed a rapid spread from China to the other countries, causing the disease that is named coronavirus disease-19 (COVID-19).[2]

Does COVID-19 cause dysregulation of autophagy?

The role of autophagy in CoV infection has been discussed before during the SARS outbreak in 2002–2003. Nowadays, two important inquiries are released: Does CoV induce autophagy or Does autophagy be dysregulated in response to CoVs infection and replication *in vivo*? The answer is still under debate.

It is thought that autophagy is involved in CoV viral replication. It was based on a study on the mouse hepatitis virus (MHV).[68] The scientists observed that MHV could induce the formation of autophagosome-like organelle (double-membrane vesicles [DMVs]), which is considered the hallmark of autophagy. They also reported that the viral replication complexes at DMVs coalesce with many proteins of autophagy like LC3 and ATG12. They also noticed that MHV replication was dysregulated in ATG5 knockout in their study. Hence, the authors concluded that autophagy could play an important pathophysiologic mechanism in the formation of autophagosome-like organelle (DMV) as well as in MHV replication.[68]

Then, the same study group investigated SARS-CoVs and its relation to autophagy. They reported a nearly similar finding. They found fusion of the key viral replication proteins with autophagosome protein marker (endogenous LC3). Hence, they suggested an intimate controlling role of autophagy on SARS-CoVs replication.[69]

In 2011, Cottam *et al*. studied another CoV strain which is called the infectious bronchitis virus. They disclosed a viral replicase protein nsp6 that can induce autophagy.[70] These study discussed the proposed pathogenic stimulant relation of CoVs of autophagy.[2,68-70] In contrast to these findings, another study documented reverse results. Snijder *et al*.[71] failed to detect any fusion of autophagy specific proteins (LC3 or GFP-LC3) with the viral replication/transcription complexes of SARS-CoV. Moreover, no autophagy protein is detected to be required for SARS-CoVs or even MHV viral replication. Hence, their observations suggested that autophagy is not directly implicated in the viral replication process.

Other controversy suggested that dysregulated autophagy mechanism is induced by COVID-19.

In 2014, a study by Chen *et al*. found overexpression of membrane-associated papain-like protease PLP2 (PLP2-TM) of SARS-CoV and MERS-CoV that is associated with blockage of autophagosomes–lysosomes fusion and consequently suppression of the autophagic flux.[72] Recently, in 2019, a study by Gassen *et al*.[73] reported also that MERS-CoV can block the fusion of autophagosomes and lysosomes. This leads to impaired induction of autophagy that leads to reduced replication of MERS-CoV.[73]

Conclusion

This review provides recent advances in understanding the types, mechanisms, and physiological roles of autophagy. Moreover, it summarizes the potential pathophysiologic contribution of autophagy dysregulation in certain pathological conditions, namely tumorigenesis, aging, age-related neurodegeneration, and endothelial dysfunctions. This article also discusses the relation between autophagy dysregulation and the pathogenesis of newly discovered CoV-COVID-19.

Financial support and sponsorship

Conflicts of interest
There are no conflicts of interest.

References

1. Keller MD, Torres VJ, Cadwell K. Autophagy and microbial pathogenesis. Cell Death Differ 2020;27:872-86.
2. Yang N, Shen HM. Targeting the endocytic pathway and autophagy process as a novel therapeutic strategy in COVID-19. Int J Biol Sci 2020;16:1724-31.
3. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, *et al*. A new coronavirus associated with human respiratory disease in China. Nature 2020;579:265-9.
4. Chun Y, Kim J. Autophagy: An essential degradation program for cellular homeostasis and life. Cells 2018;7:278.
5. Boteon YL, Laing R, Mergental H, Reynolds GM, Mirza DF, Afford SC, *et al*. Mechanisms of autophagy activation in endothelial cell and their targeting during normothermic machine liver perfusion. World J Gastroenterol 2017;23:8443-51.
6. Hassanpour M, Rezabakhsh A, Pezeshkian M, Rahbarghazi R, Nouri M. Distinct role of autophagy on angiogenesis: Highlights on the effect of autophagy in endothelial lineage and progenitor cells. Stem Cell Res Ther 2018;9:305.
7. Schaaf MB, Houbaert D, Meçe O, Agostinis P. Autophagy in endothelial cells and tumor angiogenesis. Cell Death Differ 2019;26:665-79.
8. Zhao J, Zhai B, Gygi SP, Goldberg AL. mTOR inhibition activates overall protein degradation by the ubiquitin proteasome system as well as by autophagy. Proc Natl Acad Sci U S A 2015;112:15790-7.
9. Metaxakis A, Ploumi C, Tavernarakis N. Autophagy in Age-Associated Neurodegeneration. Cells 2018;7:37.
10. Sotthibundhu A, Promjuntuek W, Liu M, Shen S, Noisa P. Roles of autophagy in controlling stem cell identity: A perspective of self-renewal and differentiation. Cell Tissue Res 2018;374:205-16.
11. Cuervo AM, Wong E. Chaperone-mediated autophagy: Roles in disease and aging. Cell Res 2014;24:92-104.
12. Wang J, Gu BJ, Masters CL, Wang YJ. A systemic view of Alzheimer disease-Insights from amyloid-beta metabolism beyond the brain. Nat Rev Neurol 2017;13:703.

13. Li W, Li J, Bao J. Microautophagy: Lesser-known self-eating. Cell Mol Life Sci 2012;69:1125-36.

14. Jiang F. Autophagy in vascular endothelial cells. Clin Exp Pharmacol Physiol 2016;43:1021-8.

15. Ruf S, Heberle AM, Langelaar-Makkinje M, Gelino S, Wilkinson D, Gerbeth C, *et al*. PLK1 (polo like kinase 1) inhibits MTOR complex 1 and promotes autophagy. Autophagy 2017;13:486-505.

16. Napolitano G, Ballabio A. Tfeb at a glance. J Cell Sci 2016;129:2475-81.

17. Mizushima N, Yoshimori T, Ohsumi, Y. The role of atg proteins in autophagosome formation. Annu Rev Cell Dev Biol 2011;27:107-32.

18. Kim J, Kundu M, Viollet B, Guan KL. Ampk and mtor regulate autophagy through direct phosphorylation of ulk1. Nat Cell Biol 2011;13:132-41.

19. Karanasios E, Stapleton E, Manifava M, Kaizuka T, Mizushima N, Walker SA, *et al*. Dynamic association of the ulk1 complex with omegasomes during autophagy induction. J Cell Sci 2013;126:5224-38.

20. Bakula D, Muller AJ, Zuleger T, Takacs Z, Franz-Wachtel M, Thost AK, *et al*. Wipi3 and wipi4 beta-propellers are scaffolds for lkb1-ampk-tsc signaling circuits in the control of autophagy. Nat Commun 2017;8:15637.

21. Altman BJ, Rathmell JC. Metabolic stress in autophagy and cell death pathways. Cold Spring Harb Perspect Biol 2012;4:a008763.

22. Wei Y, Liu M, Li X, Liu J, Li H. Origin of the autophagosome membrane in mammals. Biomed Res Int 2018;2018:1012789.

23. Johansen T, Lamark T. Selective autophagy mediated by autophagic adapter proteins. Autophagy 2011;7:279-96.

24. Zaffagnini G, Martens S. Mechanisms of selective autophagy. J Mol Biol 2016;428:1714-24.

25. Palikaras K, Lionaki E, Tavernarakis N. Mechanisms of mitophagy in cellular homeostasis, physiology and pathology. Nat Cell Biol 2018;20:1013-22.

26. Pickles S, Vigie P, Youle RJ. Mitophagy and quality control mechanisms in mitochondrial maintenance. Curr Biol 2018;28:R170-85.

27. Park C, Cuervo AM. Selective autophagy: Talking with the UPS. Cell Biochem Biophys 2013;67:3-13.

28. Puertollano R, Ferguson SM, Brugarolas J, Ballabio A. The complex relationship between TFEB transcription factor phosphorylation and subcellular localization. EMBO J 2018;37:e98804.

29. Palmieri M, Pal R, Nelvagal HR, Lotfi P, Stinnett GR, Seymour ML, *et al*. mTORC1-independent TFEB activation via Akt inhibition promotes cellular clearance in neurodegenerative storage diseases. Nat Commun 2017;8:14338.

30. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell 2008;132:27-42.

31. Yang Z, Klionsky DJ. Mammalian autophagy: Core molecular machinery and signaling regulation. Curr Opin Cell Biol 2010;22:124-31.

32. Deretic V, Saitoh T, Akira S. Autophagy in infection, inflammation and immunity. Nat Rev Immunol 2013;13:722-37.

33. McLeod IX, Jia W, He YW. The contribution of autophagy to lymphocyte survival and homeostasis. Immunol Rev 2012;249:195-204.

34. Christian P, Sacco J, Adeli K. Autophagy: Emerging roles in lipid homeostasis and metabolic control. Biochim Biophys Acta 2013;1831:819-24.

35. Singh R, Xiang Y, Wang Y, Baikati K, Cuervo AM, Luu YK, *et al*. Autophagy regulates adipose mass and differentiation in mice. J. Clin. Investig 2009;119:3329-39.

36. Maes H, Rubio N, Garg AD, Agostinis P. Autophagy: Shaping the tumor microenvironment and therapeutic response. Trends Mol Med 2013;19:428-46.

37. Rouschop KM, van den Beucken T, Dubois L, Niessen H, Bussink J, Savelkouls K, *et al*. The unfolded protein response protects human tumor cells during hypoxia through regulation of the autophagy genes MAP1LC3B and ATG5. J Clin Invest 2010;120:127-41.

38. Sormani MP. Modeling the distribution of new MRI cortical lesions in multiple sclerosis longitudinal studies by Sormani MP, Calabrese M, Signori A, Giorgio A, Gallo P, De Stefano N [PLoS One 2011;6(10):e26712. Epub 2011 October 20]. Mult Scler Relat Disord 2012;1:108.

39. Pavlides S, Vera I, Gandara R, Sneddon S, Pestell RG, Mercier I, *et al*. Warburg meets autophagy: Cancer-associated fibroblasts accelerate tumor growth and metastasis via oxidative stress, mitophagy, and aerobic glycolysis. Antioxid Redox Signal 2012;16:1264-84.

40. Lock R, Roy S, Kenific CM, Su JS, Salas E, Ronen SM, *et al*. Autophagy facilitates glycolysis during Ras-mediated oncogenic transformation. Mol Biol Cell 2011;22:165-78.

41. Kang MR, Kim MS, Oh JE, Kim YR, Song SY, Kim SS, *et al*. Frameshift mutations of autophagy-related genes ATG2B, ATG5, ATG9B and ATG12 in gastric and colorectal cancers with microsatellite instability. J Pathol. 2009;217:702-6. doi: 10.1002/path.2509. PMID: 19197948..

42. Takahashi Y, Coppola D, Matsushita N, Cualing HD, Sun M, Sato Y, *et al*. Bif-1 interacts with Beclin 1 through UVRAG and regulates autophagy and tumorigenesis. Nat Cell Biol 2007;9:1142-51.

43. Takamura A, Komatsu M, Hara T, Sakamoto A, Kishi C, Waguri S, *et al*. Autophagy-deficient mice develop multiple liver tumors. Genes Dev 2011;25:795-800.

44. Inami Y, Waguri S, Sakamoto A, Kouno T, Nakada K, Hino O, *et al*. Persistent activation of Nrf2 through p62 in hepatocellular carcinoma cells. J Cell Biol 2011;193:275-84.

45. Rubinsztein DC, Bento CF, Deretic V. Therapeutic targeting of autophagy in neurodegenerative and infectious diseases. J Exp Med 2015;212:979-90.

46. Yang F, Chu X, Yin M, Liu X, Yuan H, Niu Y, *et al*. Mtor and autophagy in normal brain aging and caloric restriction ameliorating age-related cognition deficits. Behav Brain Res 2014;264:82-90.

47. Wenz T. Mitochondria and pgc-1alpha in aging and age-associated diseases. J Aging Res 2011;2011:810619.

48. Nakamura S, Yoshimori T. Autophagy and longevity. Mol Cells 2018;41:65-72.

49. Yang L, Wang H, Liu L, Xie A. The role of insulin/igf-1/pi3k/akt/gsk3beta signaling in Parkinson's disease dementia. Front Neurosci 2018;12:73.

50. Sun L, Zhao M, Liu M, Su P, Zhang J, Li Y, *et al*. Suppression of foxo3a attenuates neurobehavioral deficits after traumatic brain injury through inhibiting neuronal autophagy. Behav Brain Res 2018;337:271-9.

51. Yu WH, Cuervo AM, Kumar A, Peterhoff CM, Schmidt SD, Lee JH, *et al*. Macroautophagy-a novel Beta-amyloid peptide-generating pathway activated in Alzheimer's disease. J Cell Biol 2005;171:87-98.

52. Elbaz A, Carcaillon L, Kab S, Moisan F. Epidemiology of parkinson's disease. Rev Neurol 2016;172:14-26.

53. Dehay B, Bove J, Rodriguez-Muela N, Perier C, Recasens A, Boya P, *et al*. Pathogenic lysosomal depletion in parkinson's disease. J Neurosci 2010;30:12535-44.

54. Koizumi K, Wang G, Park L. Endothelial dysfunction and amyloid-β-induced neurovascular alterations. Cell Mol Neurobiol 2016;36:155-65.

55. Kim ST, Tasaki T, Zakrzewska A, Yoo YD, Sa Sung K, Kim SH, *et al*. The N-end rule proteolytic system in autophagy. Autophagy 2013;9:1100-3.

56. Potente M, Gerhardt H, Carmeliet P. Basic and therapeutic aspects of angiogenesis. Cell 2011;146:873-87.

57. Menghini R, Casagrande V, Marino A, Marchetti V, Cardellini M, Stoehr R, *et al*. MiR-216a: A link between endothelial dysfunction and autophagy. Cell Death Dis 2014;5:e1029.

58. Favero G, Paganelli C, Buffoli B, Rodella LF, Rezzani R. Endothelium and its alterations in cardiovascular diseases: Life style intervention. Biomed Res Int 2014;2014:801896.

59. Vion AC, Kheloufi M, Hammoutene A, Poisson J, Lasselin J, Devue C, *et al*. Autophagy is required for endothelial cell alignment and atheroprotection under physiological blood flow. Proc Natl Acad Sci U S A 2017;114:E8675-84.

60. Ward C, Martinez-Lopez N, Otten EG, Carroll B, Maetzel D, Singh R, *et al*. Autophagy, lipophagy and lysosomal lipid storage disorders. Biochim Biophys Acta 2016;1861:269-84.

61. Torisu K, Singh KK, Torisu T, Lovren F, Liu J, Pan Y, *et al*. Intact endothelial autophagy is required to maintain vascular lipid homeostasis. Aging Cell 2016;15:187-91.

62. Ebrahim N, Ahmed IA, Hussien NI, Dessouky AA, Farid AS, Elshazly AM, *et al*. Mesenchymal Stem Cell-Derived Exosomes Ameliorated Diabetic Nephropathy by Autophagy Induction through the mTOR Signaling Pathway. Cells. 2018 Nov 22;7:226. doi: 10.3390/

cells7120226. PMID: 30467302; PMCID: PMC6315695..

63. Gödel M, Hartleben B, Herbach N, Liu S, Zschiedrich S, Lu S, *et al*. Role of mTOR in podocyte function and diabetic nephropathy in humans and mice. J Clin Invest 2011;121:2197-209.

64. Aguado C, Sarkar S, Korolchuk VI, Criado O, Vernia S, Boya P, *et al*. Laforin, the most common protein mutated in Lafora disease, regulates autophagy. Hum. Mol. Genet. 2010, 19, 2867–2876.

65. Weiss SR, Navas-Martin S. Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. Microbiol Mol Biol Rev 2005;69:635-64.

66. Schoeman D, Fielding BC. Coronavirus envelope protein: Current knowledge. Virol J 2019;16:69.

67. Zheng Y, Shang J, Yang Y, Liu C, Wan Y, Geng Q. Lysosomal proteases are a determinant of coronavirus tropism. J Virol 2018;92:e01504-18.

68. Prentice E, Jerome WG, Yoshimori T, Mizushima N, Denison MR. Coronavirus replication complex formation utilizes components of cellular autophagy. J Biol Chem 2004;279:10136-41.

69. Prentice E, McAuliffe J, Lu X, Subbarao K, Denison MR. Identification and characterization of severe acute respiratory syndrome coronavirus replicase proteins. J Virol 2004;78:9977-86.

70. Cottam EM, Maier HJ, Manifava M, Vaux LC, Chandra-Schoenfelder P, Gerner W. *et al*. Coronavirus nsp6 proteins generate autophagosomes from the endoplasmic reticulum via an omegasome intermediate. Autophagy 2011;7:1335-47.

71. Snijder EJ, van der Meer Y, Zevenhoven-Dobbe J, Onderwater JJ, van der Meulen J, Koerten HK, *et al*. Ultrastructure and origin of membrane vesicles associated with the severe acute respiratory syndrome coronavirus replication complex. J Virol 2006;80:5927-40.

72. Chen X, Wang K, Xing Y, Tu J, Yang X, Zhao Q, *et al*. Coronavirus membrane-associated papain-like proteases induce autophagy through interacting with Beclin1 to negatively regulate antiviral innate immunity. Protein Cell. 2014;5:912-27.

73. Gassen NC, Niemeyer D, Muth D, Corman VM, Martinelli S, Gassen A, *et al*. SKP2 attenuates autophagy through Beclin1-ubiquitination and its inhibition reduces MERS-Coronavirus infection. Nat Commun 2019;10:5770.