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Autophagy to Survive
Yaşamak için Otofaji

Müzeyyen İzmirli[1,2], Hasret Ecevit[2], Bülent Gögebakan[1,2]

[1]Mustafa Kemal University, Medical School, Department of Medical Biology, Hatay,Turkey
[2]Mustafa Kemal University, Medical School, Department of Molecular Biochemistry and Genetic, Hatay,Turkey

ABSTRACT
Autophagy is the catabolic mechanism that involves cell degradation of unnecessary or dysfunctional cellular components through the actions of lysosomes. It helps to keep the cells alive in such cases like oxidative stress, lack of nutrients and growth factors providing recycling of intracellular molecules. However, it works as a part of metabolism regulation, morphogenesis, cell differentiation, senescence, cell death and immune system. There are three subtype including macro-autophagy, micro-autophagy, chaperone-mediated autophagy. As a result of impairment of this mechanism, pathological situations arise including cancer, neurodegenerative and infectious diseases. Consequently, researches about autophagy mechanism are important for the development of novel diagnosis, follow-up and treatment modalities in health problems. For the first time, the review purposes to provide three subtypes of autophagy to reader.
Key words: Autophagy, Macro-autophagy, Micro-autophagy, Chaperone-mediated autophagy

ÖZET
Otofaji, gereksiz ya da disfonksiyonel hücresel komponentlerin lizozomlar aracılığıyla parçalandığı katabolik bir mekanizmadır. Oksidatif stres, besin ve büyüme faktörü yokluğu gibi durumlarda hücre içi moleküllerin geri dönüşümünü sağlayarak hücrenin hayatta kalmasına yardımcı olur. Diğer taraftan, metabolizmanın düzenlenmesi, morfogenezis, hücre farklılaşması, yaşlanma, hücre ölümü ve bağışıklık sisteminin bir parçası olarakta çalışır. Makrootofaji, mikrootofaji ve şaperon aracılı otofaji olmak üzere üç alt tipi vardır. Bu mekanizmanın bozulması neticesinde kanser, nörodejeneratif ve enfeksiyon hastalıkları gibi patolojik durumlar ortaya çıkmaktadır. Sonuç olarak, otofajik mekanizması hakkındaki araştırmalar çeşitli sağlık problemleri için yeni tanı, takip ve tedavi yöntemlerine ışık tutma potansiyeli nedeniyle çok büyük önem arz etmektedir. Bu derleme, ilk olarak, otofajinin üç alt tip

mekanizmasını birden okuyucuya sunmayı amaçlamaktadır.
Anahtar kelimeler: Otofaji, Makrootofaji, Mikrootofaji, Şaperon Aracılı Otofaji

Introduction

Autophagy (from the Greek word auto:self; phagy:eating) is a highly conserved cellular mechanism in recycling of long-lived proteins and damaged organelles[1-2]. It is a catabolic process that characterized by double membrane vesicles named autophagosomes that converge with lysosomes after engulfing intracellular macromolecules and organelles for degrading these structures to recycle the building blocks to the cell again[3].

Keith R. Porter firstly realized the process of autophagy in Rockefeller Institute. In 1962, Porter et al. reported unknown structures that increased number, displaced toward the center of the cell and containing organelles like mitochondria in liver cells of rats given glucagon. This was the first evidence about the intracellular digestion of organelles. However, Porter et al. thought that lysosomes weren't intracellular organelles and hydrolytic enzymes were produced by peroxisomes[4]. Duve, who originated is the Nobel Prize-winning discoverer of lysosomes and peroxisomes, proposed that glucagon was the major stimulant of lysosomal function by inventing the term autophagy, unlike Porter et al. Then, Russell L. Peter et al. determined that lysosomes are the responsible organelles about glucagon-induced autophagy[5,6].

It was shown that autophagy mechanism help the cell to exceed for stress environment like oxidative stress or lack of nutrients and growth factors and allows recycling of intracellular macromolecules in earlier studies about autophagy. Thereby, it provides the protection of the cell homeostasis[7,8]. In studies in the past decade, it was determined that autophagy is an effective mechanism in metabolism regulation, morphogenesis, cell differentiation, aging, cell death and as a part of the immune system in the destruction of intracellular pathogens[2,9]. As a result of degeneration of this mechanism and homeostasis, neurodegenerative diseases, pathological conditions such as cancer and infectious diseases arise[10].

Autophagy is divided into three categories according to the route of cargo (organelles, proteins) delivery; macro-autophagy, micro-autophagy and chaperone-mediated autophagy[11] and we aim to firstly assess mentioned subtypes of autophagy.

Macro-autophagy

Macro-autophagy basically starts by the formation of structures called autophagic vesicles and these structures are added end to end to form autophagosome. When there is a stress condition (for example; starvation), long-lived proteins or organelles are enclosed by autophagic vesicles and they form autophagosomes. After this stage, autophagosomes merge with lysosomes and their contents are degraded by lysosomal enzymes. In this way, subunits including amino acids and fatty acids are created for re-use within the cell[12].

In macro-autophagy, class III phosphoinositol 3-phosphate (PI3F) kinase is one of the structure which takes part in autophagosome formation. This enzyme directs the protein groups that bound itself to the preautophagosomal structure (PAS). In this way, autophagosome membrane seed forms. The elongation and taking the form of vesicle of this membrane seed is occurs via two ubuquitin-like systems. The first one is characterized by covalent binding of Atg12 to Atg5. Then, Atg12-Atg5 complex combine with Atg16. This oligomer (Atg5-Atg12-Atg16) connects with the outer surface of isolation membrane[12]. In the second system, Atg8 protein (mammals Microtubule Associated Protein Light Chain 3; MAP-LC3) is covalently linked to a fatty molecule. To achieve this connection, Atg12-Atg5-Atg16 must be formed. In addition, Atg4 protease must cut five amino acids which are at the C-terminal of Atg8/LC3 to expose the sixth amino acid glycine. Because, glycine is the place that phosphatidylethanolamine (PE) will connect[1,2]. By providing this connection, membrane moved to the PAS and elongation of the membrane is provided. After the formation of autophagosome, Atg4 pick off LC3 proteins from fatty molecule and provides the re-use. Formed autophagosome connects with lysosome and lysosomal enzymes degrade the autophagosome contents. Finally, degraded contents are imparted to the cell for re-use[12,13].

Micro-autophagy

Micro-autophagy is the autophagic process that lysosomal membrane invaginates randomly and differentiates as an autophagic tubule to surround the cytosolic fractions. Then this tubule merge with lysosomal lumen and tubular cargo is degraded. Duve and Wattiaux had shown macro-autophagy and micro-autophagy in 1966 in rat liver however the difference between macro-autophagy and micro-autophagy had appeared in 1983s[14,15].

In the past two decades, the growth in our understanding about micro-autophagic process almost all has come from studies with yeast (*Saccharomyces cerevisiae*, *Pichia pastoris, Hansenula polymorpha*). Unlike the macro-autophagic process that autophagosomes containing the cytosolic fractions is fused with lysosomes and morphologically more open, micro-autophagy is characterized by the local deformation of lysosomes to engulf the cytoplasm or any component parts. Few investigators have studied micro-autophagy in mammalian cells as a primary focus, so our understanding has remained limited[16-18].

In the early stage of micro-autophagy, lipids laterally decomposes, big transmembrane proteins are excluded from the membrane and consequently lysosomal membrane bulge into the lumen. Independent of the intracellular environment, certain lipids and lipid-modifying proteins drive and maintain spontaneously a cavity. A dynamin-related GTPase Vps1p regulates micro-autophagic invagination[19]. The frequency of invaginations depends on the nutritional conditions. For example, starvation induces the initiation of the invagination. After bulging, the invagination extends and specializes as a shape termed autophagic tubule[20]. ATP is necessary for these steps[21]. Micro-autophagy is accompanied by two Atg7-dependent ubuquitin-like conjugation systems (Ub1c; Ubuquitin like conjugation). In the first Atg7-Ub1c system, a cysteine protease Atg4, provides the Atg8-phosphatidylethanolamine connection. In the second Ub1c system, Atg7 (E1-like enzyme) and Atg10 (E2-like enzyme) bond Atg12 to Atg5. Atg12-Atg5 dimer oligomerizes with Atg16 to stimulate the formation of Atg8-phosphatidylethanolamine. The two Ub1c systems organize the vesicle formation and expansion[22-24].

In addition to the Atg7-dependent Ub1c, vacuolar transporter chaperone (VTC) complex plays a vital part in the tube organization of yeast[19]. Under stress condition like nutrient limitation, VTC complex that consist of Vtc1p, Vtc2p, Vtc3p and Vtc4p controls the distribution of membrane proteins in different compartments[20].

Micro-autophagy is induced by GTPase and membrane potential, is ATP and Mg dependent[21]. V-ATPase acidifies the lumen by pumping H to create an electrochemical gradient. This event is necessary for membrane fusion[25].

Studies showed that there are two mechanism of micro-autophagy; nonselective autophagy and selective autophagy in yeast[18].

After the seperation of autophagic tubes, free vesicles move around in the lumen at a high speed. Atg15p and other hydrolyses break down the vesicle and then Atg22p acts as a permease for the recycling of nutrients and energy[26,27].

Nonselective Micro-Autophagy

Nonselective micro-autophagy is the type of micro-autophagy that characterized by lysosomal membrane invagination which surrounds the cytosolic structures will be degraded18.

Selective Micro-Autophagy

In yeast, several subtypes of microautophagy that is special for autophagic cargo (mitochondria, nucleus and peroxisome) were defined. It is named micomitophagy, micronucleophagy, micropexophagy (respectively for, mitochondria, nucleus, peroxisomes)18.

Chaperone-Mediated Autophagy

Chaperone-mediated autophagy is a catabolic process that cytosolic proteins having KFERQ-like (KFERQ: K, lysine; F, phenylalanine; E, glutamic acid; R, arginine; Q, glutamine) motif are translocated to lysosome membrane and then into the lysosome by chaperone-dependent selection and degraded. In this process, unlike micro-autophagy and macro-autophagy, substrate protein can be taken into the lysosome and degraded without any vesicle formation[28].

The basis of chaperone-mediated autophagic mechanism relies on a penta peptide motif (five peptide), KFERQ-like signal sequence which is present in 30% of cytosolic proteins. This motif consists of Q preceded or followed by four amino acids, a basic, an acidic, a bulky hydrophobic and a repeated basic or bulky hydrophobic amino acid. Most of the substrates known about chaperone-mediated autophagy biochemically contain this signal sequence (KFERQ)[28]. KFERQ signal sequence is recognized by another structure of chaperone-mediated autophagy 'molecular chaperone complex'. This complex that consists of many subunits, carries the substrate protein to the next step which is at the lysosomal membrane (LAMP-2A)[29]. The subunits of the complex are; hsc70 (heat shock cognate protein; 70kDa), hsp40 (heat shock protein; 40 kDa), hsp 90 (heat shock protein; 90 kDa), Hsc70 interacting protein (hip : heat), Hsc70-hsp90 organizer protein (hop: heat organizer protein) and Bcl2‑associated

athanogene‑1 (bag‑1). Hsc70 stimulates the translocation of protein from lysosomal membrane[30-33] and recognize the KFERQ signal sequence at the substrate protein[29]. Hsp40 is another molecular chaperone that provides the ATPase activity of Hsc70 and regulates substrate connection. The co-chaperones that interact with hsc70 act as chaperones themselves or regulate the activities of hsc70[32]. Hip stimulates the montage of hsc70 with hsp40 and substrate protein[30]. Hsp90 recognizes the unfolded proteins and prevents substrate aggregation. Hop provides the link between hsc70 and hsp90[28]. Bag-1 regulates the activity of hsc70 as a positive or negative regulator[33].

Molecular chaperone complex delivers the substrate will be degraded to another component at the lysosomal membrane called LAMP-2A. LAMP2 (Lysosome-associated membrane protein 2) or CD107b (Cluster of Differentiation 107b) is a human gene. The protein that encoded by this gene is a member of a family of membrane glycoproteins which provides selectins with carbohydrate ligands. It may play a role in tumor cell metastasis, function in the protection, maintenance, and adhesion of the lysosome. The gene produces three variants LAMP-2A, LAMP-2B and LAMP-2C. LAMP-2A is the receptor for chaperone-mediated autophagy[34]. Substrate connects with LAMP-2A at the lysosomal membrane and translocated to the lumen for degrading[28]. Studies showed that lysosomal hsc70 (ly-hsc70) is also necessary for translocation[34].

To summarize the chaperone-mediated autophagic mechanism;

1. Molecular chaperone complex that consists of hsc70, hip, hop, hsp40, hsp90 and bag-1 recognize the KFERQ signal sequence on the substrate protein.
2. Molecular chaperone-substrate complex connects with multisubünit form of LAMP-2A at the lysosomal membrane.
3. Substrate protein must be unfolded before the translocation.
4. Lysosomal hsc70 (ly-hsc70) is also necessary for translocation.
5. Substrate protein degrades by lysosomal proteases in the lysosomal lumen.
6. Hsc70 chaperone complex leaves the lysosomal membrane.
7. Hsc70 that leaved the lysosomal membrane now can bind to a new substrate protein[29].

Conclusion

Autophagy is an important process for survival of cell, metabolism regulation, morphogenesis, cell differentiation, aging, cell death and immune system. If these mechanisms degenerate, lots of pathologies can arise, for example cancer, neurodegenerative and infectious diseases. Consequently, the clarification of this mechanism that sometimes provides cell survival under stress conditions and sometimes fatal is of great importance in terms of providing the potential of new diagnosis, and treatment methods. Thus, we think that new researches will be useful to clarify the issue.

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Correspondence Address / Yazışma Adresi
Müzeyyen İzmirli
Mustafa Kemal University, Medical School,
Dept of Medical Biology and Molecular Biochemistry and Genetic
Hatay, Turkey
e-mail: muzeyyenizmirli@gmail.com