Autophagy: Self Eating Process

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Abstract

Autophagy is a catabolic, naturally occurring cellular mechanism for degradation and recycling of cellular components. The process is mediated by the formation of a double membrane structure –the autophagosome. This review includes the regulation of autophagy, mechanism comprises of initiation, formation, membrane expansion, and fusion with lysosome. Regulatory pathway of autophagy is a complex one and associated with various proteins and metabolic pathways. mTOR plays a key role in regulation with the involvement of kinase like AMPK which is sensitive to cellular energy level (ATP) and the involvement of other regulatory proteins, TSC dependent or independent pathway in autophagy regulation is described here. Along with this growth factor and cellular stress signals in autophagy regulation are also included. Role of autophagy in maintaining cellular homeostasis and significance of autophagy in suppression of tumerogenesis along with recent development is discussed. Also the relation of autophagy in Alzheimer's disease, infections has been highlighted briefly.

Keywords: *Autophagosome, ATG, mTOR, ULK kinase*

INTRODUCTION

Autophagy (Greek, autophagos-self devouring) , term was coined by Christian de Duve (Nobel prize in physiology or medicine in 1974 for discovery of lysosomes). Autophagy commonly also referred as macroautophagy is a process by which the cellular components are degraded and recycled by the formation of autophagosome. It frequently comes into play during starvation, allowing normal cells to survive. Yoshinori Ohsumi and coworkers observes morphological changes in the vacuoles of starving yeast cells, many vesicles were accumulated and the autophagosome formation and fusion with vacuole was seen using electron microscope. His students found the first autophagy defective mutant atg1 (initially named as apg 1), later they also found other 14 atg mutants [1].With the discovery of autophagy related proteins the mechanism behind autophagy became more clear and this important cellular process came into focus.

The cellular and molecular pathway of this cycle is conducted by various autophagy related proteins (Atg). Many autophagies related (Atg) genes which products regulate autophagy have been identified and studied primarily through the use of yeast [2]. Many research shows the stress-induced birth of the autophagosome after amino acid starvation in yeast and in mammalian cells[3].This unique cellular process is known to prevent the gradual accumulation of damaged proteins and organelles in cells that is toxic for cell as time expands, thus autophagy plays an important

role in maintaining quality of cells by acting as a cell garbage cleaner [4]. Also this evolutionarily conserved pathway is a significant regulator of many cellular metabolisms, which deregulation lead to various human diseases.

Unlike in macroautophagy , in other category which are microautophagy and chaperon mediated autophagy autophagosome formation does not takes place. In microautophagy lysosome is directly involved in degradation of cellular materials by direct entry through an invagination. The charperon mediated autophagy is involved in the protein degradation by utilizing chaperone HSC70 /HSP8A and directly translocate them into the lysosome[5] . Among the three macroautophagy is the most prominent one [6] and is detailed in this review.

Stress Autophagy can also be distinguished into non-selective and selective types. Non-selective autophagy is involved in degradation of bulk cytoplasm randomly under starvation condition. Mitophagy, Pexophagy and xenophagy are selective autophagy involved in selective degradation of damaged mitochondria, peroxisomes and microbes respectively[7,15].

REGULATION OF AUTOPHAGY

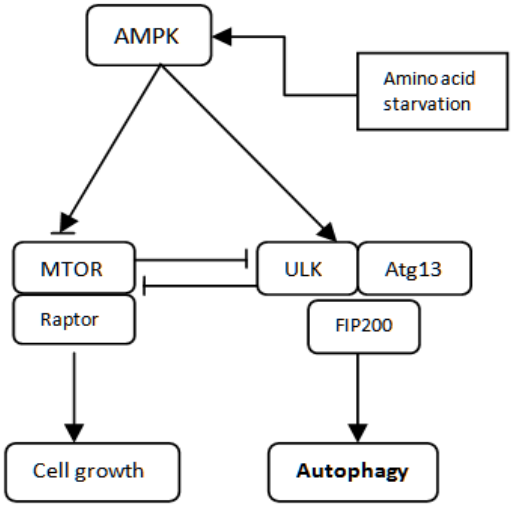

Fig:1 Amino acid starvation induce autophagy pathway While mTOR(cell growth) is inhibited

In the cellular homeostasis various cellular proteins are involved and are stimulated or inhibited depending upon availability of nutrients, growth factors e.t.c. and ultimately switch cell towards anabolic or catabolic pathway; autophagy is one of the catabolic pathway. mTOR (mammalian target of rapamycin) acts as a negative regulator of autophagy [8] and various proteins like PTEN,PDK1,Akt,TSC1/2 are involved upstream of mTOR signalling for its regulation. PTEN and TSC1/2 positively regulate autophagy whereas Akt inhibit it[9,10]. PTEN acts by blocking class I phosphatidylinositol (PtdIns) 3-kinase to induce autophagy. Since upstream of mTOR PtdIns 3-kinase is involved in inhibitory cascade of autophagy. For the balance between growth and autophagy, mTOR belonging to the phosphatidylinosotol kinase-related kinase family (PIKK) is a key component involved. mTOR acts as growth promoter by inducing growth promoting cascade involving regulatory associated protein of mTOR (Raptor) and by inhibiting ULK (serine/threonine kinase complex) when sufficient nutrients are available. The ULK (serine/threonine kinase complex) is one of the important components involved in autophagy induction of mammalian cells and are highly regulated by mTOR and AMPK. ULK kinase

AMPK	+	AUTOPHAGY
	-	CELL GROWTH
ULK	+	AUTOPHAGY
mTOR	+	CELL GROWTH
GROWTH FACTOR	+	CELL GROWTH
CELLULAR STRESS/HYPOXIA	+	AUTOPHAGY

Regulatory components & their effect
+ (activation), - (inhibition)

forms a complex with ATG proteins and FIP200 (focal adhesion kinase family interacting protein of 200 kD) and propel the system towards cell degradation pathway. AMPK (AMP activated protein kinase) acting upstream of mTOR promotes autophagy during starvation (fig1) by sensing cellular energy (AMP:ATP ratio) [11]. Activation of AMPK caused by reduced ATP level due to starvation inhibits mTOR through phosphorylating and activating TSC2 (Tuberous sclerosis complex 2).TSC2 is a negative regulator of mTOR. Also by phosphorylating raptor at ser863 position AMPK can inhibit mTOR(complex 1) pathway independent of TSC2. AMPK In non starvation condition when amino acid/nutrient is sufficient some growth factors and nutrient signalling activate mTOR and it acts as anabolic switch and promotes cell growth because mTOR activity is regulated by amino acid and glucose levels in mammalian cells. Under low glucose level it has ben proposed that glyceraldehydes-3-phosphate dehydrogen-ase also conveys inhibitory signal to mTOR and is independent of TSC 1/2 pathway [12,13]. In the negative regulation of autophagy growth factors is also one of the significant contributor. Insulin/insulin like growth factor(IGF-1) act by positively regulating mTOR pathway with the involvement of PDK1 and Rheb(Ras homolog enriched in bran).During cellular stress/hypoxia autophagy undergoes induction by negative regulation of mTOR via REDD1 protein (regulated in development and DNA damage 1)[12].

MECHANISM OF AUTOPHAGY

Initiation

As already discussed in the regulation of autophagy, that the initial signal for the autophagosome formation is the amino acid starvation/nutrient deprivation /cellular and begins with the ULK kinase complex activation[3]. Atg 1 forms a complex with Atg 13, Atg 17, Atg 29 and Atg 31 upon induction of autophagy in yeast while in mammals the functional homologue of Atg 1 the ULK kinase(serine/threonine protein kinse) ULK 1 & ULK 2 forms complex with Atg 13,FLP 200 and Atg 101 [14]. The ULK1/2 complex then activates Beclin-Vps34 (PI3K3) complex via phosphorylatation[13].

Formation

Omegasome, which is a lipid bi-layer omega shaped membrane starts forming to endoplasmic reticulum (ER) subdomains[15]. ER that has been mark by Atg 9, with involvement of the class 3 phosphatidylinositol 3-kinase follows generation of the structures and is further extended[3]. Autophagosome formation is known to reuse phosphatidylinositol 3-phosphate(PtdIns3P), since it is involved in recruitment of some ATG components and occurs near the ER[2,16].The supply of lipid to the growing membrane in mammalian cell is suggested to come from Endoplasmic reticulum exit site(ERES), mitochondria, ER-Mitochondria contact site(ERMCS) , ER-Golgi intermediate compartment(ERGIC), Golgi bodies and plasma membrane but the exact mechanism is not clear ,and their contribution in different phase of autophagy is shown in fig 2 [19].

Membrane Expansion

Here omegasome membrane is expanded to from phagophore, it starts enclosing the cytoplasmic components and with the involvement of various proteins, formation of autophagosome takes place.
Autophagosome formation requires two ubiquitin like conjugation system the Atg 12 and Atg 8 system which are associated with expansion of autophagosomal membrane[16], also Atg12-Atg5 conjugate system are localised to the pre-autophagosomal structures assembly site and dissociated after achieving

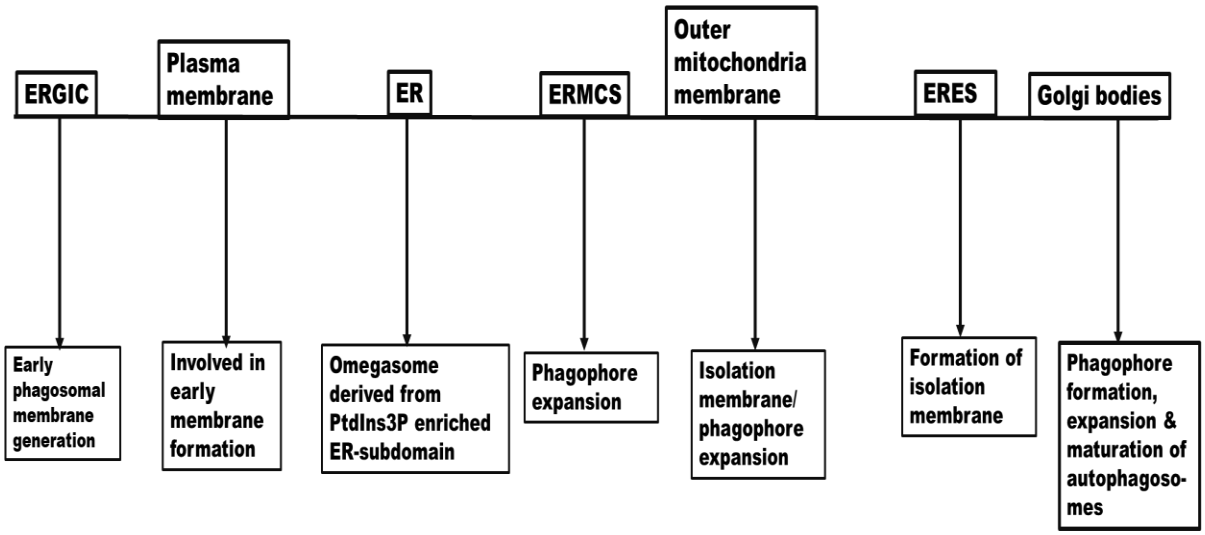

Fig 2. Cellular components and their functions in autophagy

autophagosome formation[18] . Activation of various proteins takes place and Atg protein complex are formed which undergo membrane expansion and the autophagosome finally becomes ready for fusion with lysosome [9,19].

Autophagosome-Lysosome Fusion

Mature autophagosome gets fuse with lysosome to form autolysosome or autophagolysosome – the degradative autophagic vacuole [20, 21] then the inner autophagic membrane and inner content of autophagosome are digested by hydrolytic enzymes of lysosome since lysosomes are the main degradative compartments in mammalian cells[22]. After degradation the resultant small molecules mainly amino acids are transported back to the cytoplasm for various cellular functions.

The autophagosome and lysosome must first move closer together for fusion and the cytoskeleton is involved in the movement of autophagosomes and which is a bidirectional movement. Autophagosome can be formed in any region of cytoplasm randomly but they have to move towards the perinuclear region because late endosomes and lysosomes are predominantly found in those regions. The mature autophagosomes move along microtubule tracks towards the lysosomes, located near nuclear region[23] and is mediated by Rab7 which binds to RILP(Rab interacting lysosomal protein) and ORP1L in order to mediate dynein and/or dynactin –driven movement towards the perinuclear region[24]. Also the Involvement of SNARE proteins in yeast autophagosome-vacuole fusion has been established [18].

SIGNIFICANCE:

Autophagy is involved in maintenance of cellular homeostasis and genomic integrity by degrading aged or malfunctioning cellular organelles, cytoplasm & proteins. Experimental evidences shows that autophagy sustains cell survival during nutrient deprivation by producing energy through catabolism, but also that autophagy is a means of achieving cell death when process is completed[25].

Defects in autophagy are associated with increased tumorigenesis, and the mutation of various autophagy related gene has been observed in various human cancer [26]. Various studies suggest that the mechanism behind autophagy is not only involved in cancer suppression but also involved in cancer progression. Autophagy can protect against development of cancer but in an established tumor it can support its growth and progression [27], thus detail understanding at molecular

level is needed. Although therapeutic approach utilizing autophagy pathway were experimented and some are in progress, for example- Huganpian (a traditional Chinese medicine) for liver cancer[28], miRNA regulated autophagy in colorectal cancer[29].

As autophagy is involved in the removal of amyloid deposit to some extent, the dysfunction of autophagy is suggested to lead the gradual accumulation of noxious proteins in the Alzheimer's disease (AD)[30]. New studies in transgenic mouse model also conform that autophagy is involved in removal of soluble and aggregated forms of tau. Malfunctioning of neuronal autophagy is not only involved in AD but also leads to other neurodegenerative disorders including Parkinson's disease, Huntington's disease. By inhibiting AKT/MTOR pathway and activating AMPK pathway autophagy is induced causing amyloid deposits removal, Arctigenin (extract from Arctium lappa) acts by this mechanism[31].

Autophagy is also involved in selective delivery of microorganisms to lysosomes for degredation [32] thus it has some role in infection eradication

CONCLUSION

As various proteins are involved in the process of autophagy the detail understanding of this complex pathway at the molecular level to establish the role of autophagy in various cellular pathway and diseases is need of today for developing successful therapy in future. The balance between MTOR pathway and autophagy pathway (anabolism and catabolism) is important for maintaining cellular homeostasis. Loss of regulation between the two pathways may lead to serious consequences like tumorigenesis. Autophagy in suppression of tumorigenesis and other studies suggesting the protection of tumor cells from cell death by autophagy needs more research to develop clear concept regarding mechanism in regulation of cancer. Also several neurological and immunological disorders need to be focused in relation to autophagy for successful attempt on development of therapy.

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