Seminars in Cancer Biology 23 (2013) 361–379

Contents lists available at ScienceDirect

Seminars in Cancer Biology

journal homepage: www.elsevier.com/locate/semcancer

Review

Autophagy regulation and its role in cancer

Séverine Lorin[a], Ahmed Hamaï[b], Maryam Mehrpour[b], Patrice Codogno[b,*]

[a] UPRES EA4530, Université Paris-Sud, Faculté de Pharmacie, 5 rue Jean-Baptiste Clément, 92296 Châtenay-Malabry cedex, France
[b] INSERM U845, Université Paris Descartes, 96 rue Didot CS61431, 75993 Paris cedex 14, France

ARTICLE INFO

Keywords:
Macroautophagy
Tumorigenesis
Signaling pathways
Tumor suppression
Oncogenes

ABSTRACT

The modulation of macroautophagy is now recognized as one of the hallmarks of cancer cells. There is accumulating evidence that autophagy plays a role in the various stages of tumorigenesis. Depending on the type of cancer and the context, macroautophagy can be tumor suppressor or it can help cancer cells to overcome metabolic stress and the cytotoxicity of chemotherapy. Recent studies have shed light on the role of macroautophagy in tumor-initiating cells, in tumor immune response cross-talk with the microenvironment. This review is intended to provide an up-date on these aspects, and to discuss them with regard to the role of the major signaling sub-networks involved in tumor progression (Beclin 1, MTOR, p53 and RAS) and in regulating autophagy.

1. Introduction

The word autophagy, from the Greek *self-eating*, refers to the catabolic processes through which the cell recycles its own constituents [1]. The proteasome is also involved in cell degradation, but the term *autophagy* is used solely to refer to the pathways that lead to the elimination of cytoplasmic components by delivering them into lysosomes. To date, three major types of autophagy have been described: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) [1,2]. This review will focus on macroautophagy (hereafter referred to simply as autophagy), because the evidence that the other forms of autophagy play any role in tumor biology is relatively limited [1]. Macroautophagy starts with the formation of a doubled-membrane bound vacuole, known as the autophagosome, that engulfs fractions of the cytoplasm in an either unselective or selective manner via the activity of the autophagy adaptors (SQSTM1/p62, NBR1, NDP52 and optineurin) that form a bridge between the target and the growing autophagosome membrane [1,2]. After being formed, most autophagosomes receive input from the endocytic vesicles to form an amphisome, in which the autophagic cargo is degraded and delivered into the lysosomal lumen [1]. At its basal rate, autophagy exercises quality control of the cytoplasm of most cells by removing damaged organelles and protein aggregates [1–3]. Autophagy responds to a range of stimuli, and in most cases protects cells against stressful situations [1–3]. In response to starvation, autophagy is important for the lysosomal recycling of metabolites to the cytoplasm, where they are reused either as source of energy or to provide building blocks for the synthesis of new macromolecules.

The discovery of ATGs (autophagy-related genes) in eukaryotic cells, and that of the role of ATG proteins in the formation of autophagosomes were milestones in the understanding of the molecular aspects of autophagy, and of the source of the membrane involved in the assembly of ATG proteins to form the phagophore, the isolation membrane that subsequently elongates to form the autophagosome [2,4,5]. At a molecular level, the first step in the initiation of autophagy is the activation of a molecular complex containing the serine/threonine kinase ULK1 (the mammalian ortholog of Atg1 in yeast) [4]. The activation of this complex is down-regulated by MTORC1, which integrates multiple signaling pathways that are sensitive to the availability of amino acids, ATP, growth factors, level of ROS. The expansion, curvation and closure of the autophagosome are controled by another molecular complex containing phosphatidylinositol 3-kinase (PI3K) and Beclin 1 (the mammalian orthologue of Atg6 in yeast), which allows the production of phosphatidylinositol 3-phosphate (PI3P) to occur, and the subsequent recruitment of PI3P-binding proteins WIPI1/2 [6] and two ubiquitin-like conjugation systems ATG12–ATG5-ATG16L and LC3-PE. The final fusion with lysosome requires small Rab GTPases and the transmembrane protein LAMP2 [7]. Acid hydrolases and the cathepsins present in the lysosomal lumen degrade the autophagosomal cargoes.

Advances in our understanding of the autophagic process paved the way for the discovery of the importance of autophagy in development, tissue homeostasis, metabolism, the immune response and various disease [2,8,9]. Interest in the role of autophagy in cancer stems from the discovery that *BECN1* (the gene that encodes Beclin 1) is also a haplo-insufficient tumor suppressor gene [10]. In fact, it appears that autophagy is under the control of a large panel of

* Corresponding author. Tel.: +33 0172606476; fax: +33 0172606399.
E-mail addresses: severine.lorin@u-psud.fr (S. Lorin), ahmed.hamai@inserm.fr (A. Hamaï), maryam.mehrpour@inserm.fr (M. Mehrpour), patrice.codogno@inserm.fr (P. Codogno).

1044-579X/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.semcancer.2013.06.007

oncogenes and products of tumor suppressor genes [11,12]. However, the role of autophagy in tumors is complex and ranges from a tumor suppressive role to a role in adapting to the environment [13–16]. This review will summarize what we know about the various aspects of autophagy in cancer, and present the emerging role of autophagy in cancer stem cells, in cancer cell dormancy and in the cross-talk between cancer cells and the microenvironment.

2. Regulation of autophagy by tumor suppressors and oncogene networks

Autophagy is under the control of tumor suppressors and oncogenes. Tumor suppressors have a stimulatory effect on autophagy, whereas oncogenes down-regulate it. In this section, we focus on two tumor suppressor networks (MTOR and Beclin 1) that control the very early stage of autophagy. We also discuss the role of p53 and RAS in autophagy, because the role of these proteins in autophagy is context- and location-dependent (Fig. 1). Readers interested in a more detailed discussion of the role of tumor suppressors and oncogenes in the regulation of autophagy should see several recent reviews [11–13].

2.1. The MTOR network

The mammalian (or mechanistic) target of rapamycin (MTOR), an evolutionarily conserved serine/threonine kinase, may serve as the main down-regulator of autophagy in cancer cells (for reviews, see [17,18]). The role of MTOR is not restricted to the control of autophagy; its areas of action include the regulation of protein synthesis, microtubule organization, lipid biogenesis, and cell cycle progression depending upon growth factor and nutrient availability [19,20]. Many signals, such as growth factors, genotoxic stress, amino acids, glucose, oxygen, and energy status, regulate the MTOR pathway [21]. MTOR interacts with many proteins to form at least two distinct multiprotein complexes: MTOR complex 1 (MTORC1) and MTOR complex 2 (MTORC2) [22]. These complexes are involved in regulating autophagy. MTORC1 and MTORC2 have both shared (MTOR, mLST8 and Deptor) and distinct components. Raptor and PRAS40 are unique to MTORC1, whereas rictor, mSin1, and Protor are specific to MTORC2. mLST8 and the recently identified Deptor are up- and down-regulators, respectively. MTORC2 regulates autophagy via Akt-FoxO3 in skeletal muscle cells in response to fasting conditions [23,24].

There are at least three main signaling pathways that regulate MTORC1, including RAS-proto-oncogene and the class I PI3K-AKT pathway that activate MTORC1 whereas the liver kinase B1 (LKB1) AMP-activated protein kinase (AMPK) pathway can inhibit MTORC1 [25].

The tumor suppressor “Phosphatase and TENsin homolog deleted on chromosome Ten” (PTEN) inhibits the class I PI3K-AKT pathway, thus stimulating autophagy via PtdIns (3, 4, 5) P3-phosphatase activity, thereby antagonizing the insulin/IGF pathway [26,27].

Several kinases, including calcium/calmodulin kinase kinase-β (CAMKK β), which is activated by cytosolic Ca^2, and TGF-β-activated kinase-1 (Tak-1, which is involved in IKK activation), can activate AMPK by phosphorylating a threonine residue on its catalytic α-subunit [28]. Thus, induction autophagy is also dependent on the inhibition of MTORC1 by AMPK in non-starved cells in response to an increase in free cytosolic Ca^{2+} [29]. AMPK also activates autophagy in the colon cancer cell line HCT116, which is under the control of tumor suppressor p53 [30]. The three pathways (RAS, class I PI3K-AKT, AMPK) may converge upstream of MTORC1 at the tuberous sclerosis protein 1/2 (TSC1/TSC2) complex, which is a known tumor suppressor. Interestingly, mice that are doubly heterozygous for TSC2 and PTEN have more active Akt and display faster tumorigenesis in some organs than singly heterozygous mice [31,32]. Under normal conditions, TSC1 and TSC2 form a dimer and function as a GTPase-activating protein to inactivate the RAS homolog enriched in brain (Rheb), thus down-regulating MTORC1 activity [33]. Energy deficiency, genotoxic stress, and oxygen deprivation all promote TSC1/TSC2 action and inhibit MTORC1, whereas growth factors block TSC1/TSC2 activity and activate MTORC1. Alternatively, amino acids can activate MTORC1 through the action of the Rag GTPases. In the presence of amino acids, the Rag GTPases interact with MTORC1, which promotes the translocation of MTORC1 from the cytoplasm to the lysosomal membranes, where Rheb is localized [34]. Recently it has been shown that MTORC1 senses lysosomal amino acids in a vacuolar H+ ATPase-dependent manner [35]. In addition to amino acids, glucose is also able to signal to the Rag GTPases to control the activity of MTORC1 [36].

Activated class I PI3K, through the conversion the phosphatidylinositol-4,5-biphosphate (PIP2) to phosphatidylinositol-4,5-triphosphate (PIP3), phosphorylates and activates Akt. Phosphorylated Akt in turn phosphorylates and destabilizes TSC2, leading to TSC1/TSC2 disruption, thus abolishing the down-regulatory effect of the TSC1/TSC2 complex on MTORC1, thereby inactivating autophagy [37]. In contrast, the phosphorylation of TSC2 by AMPK increases its GTPase activity, stabilizes the TSC1/TSC2 complex, inactivates Rheb, and leads to the inactivation of MTORC1; thereby activating autophagy.

In mammals, two orthologs of yeast Atg1, known as uncoordinated 51-like kinase 1 (ULK1) and ULK2, have been linked to starvation-induced autophagy. Both are found in a stable complex with mammalian autophagy-related protein 13 (mAtg13) and the 200-kDa, scaffold protein FAK-family interacting protein (FIP200) which is the functional homolog of the yeast Atg17, and Atg101, an additional binding partner of Atg13. By phosphorylating ULK and mAtg13 in a manner regulated by nutrient starvation, MTORC1 disrupts the binding of mAtg13 to ULK and destabilizes ULK, inhibiting the ULK-dependent phosphorylation of FIP200 and inducing autophagy [24,38]. Alternatively, ULK1/2 is phosphorylated by AMPK and thereby activated [39–41]. It should be noted, however, that some reports suggest that MTORC1 is incorporated into the ULK1:Atg13:FIP200 complex via ULK1 in a nutrient-dependent manner. Under starvation conditions or in response to an increase in the ratio of AMP/ATP (a condition that activates AMP-dependent kinase) or in response to rapamycin treatment, MTORC1 dissociates from the ULK1 complex, resulting in the activation of ULK1 [42]. Moreover, MTORC1 regulates autophagy by mediating protein translation and cell growth through the phosphorylation of the inhibitor of translation initiation (4E-BP1) and that of polypeptide 1 ribosomal protein S6 kinase-1 (p70S6K) [43]. It should be noted that p70S6K activity can be downregulated by ULK1, ULK2 and Atg13, indicating that the existence of a positive-feedback loop may enhance nutrient-dependent autophagy [24]. Moreover, activation of MTORC1 is required for the termination of starvation-induced autophagy and lysosomal reformation [44]. Thus, the activation and inhibition of MTORC1 is a fine tuning mechanism that regulates the initiation and termination of autophagy. Lastly, it should noted that numerous oncogenes and tumor suppressors are incorporated into the MTORC1-centered signaling network, suggesting a close, albeit complex connection between these oncogenes and tumor suppressors in the autophagic pathways of cancer (Fig. 1).

2.2. The Beclin 1 network

Beclin 1, which shares 24.4% identity with the yeast Atg6, has been shown by a two-hybrid screen to be a Bcl-2 interacting protein