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Chapter 2

Interconnected Regulation of Apoptosis and WIPI-Mediated Autophagy

Katharina Sporbeck, Fenja Odendall and Tassula Proikas-Cezanne

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Abstract

Autophagy is a genetic program that secures the survival of eukaryotic cells to compensate for periods of starvation and cellular stress. Apoptosis, in contrast, is a genetically defined program leading to cell death. Both pathways are interconnected through conserved co-regulatory signaling pathways that are context-dependent. Proper co-regulation of autophagy and apoptosis, whereby autophagy exerts an antiapoptotic function and apoptosis inhibits autophagic survival strategies, critically secures the survival of healthy cells and counteracts genomic instability in eukaryotic organisms. Cancer cells often become resistant to apoptosis and addicted to autophagy, making this scenario highly relevant to define novel therapeutic strategies. The co-regulatory crosstalk between apoptosis and autophagy converges on the production of phosphatidylinositol 3-phosphate (PI3P) essential for the onset of autophagy. WD-repeat protein interacting with phosphoinositides (WIPI) members function as essential PI3P effectors in autophagy and fulfill an important role in health and disease. Here, we summarize details on the regulation of WIPI-mediated autophagy in the context of co-regulatory signals for both apoptosis and autophagy.

Keywords: WIPI, PI3P, autophagy, autophagosomal cell death, p53

1. Introduction

The process of autophagy was conceptualized in 1963 by Christian de Duve, who coined the term based on the Greek words *auto* for self and *phagy* for eat, hence self-eating. de Duve suggested that intracellular vesicles, derived from the endoplasmic reticulum, harbor cytoplasmic material for lysosomal degradation. This novel concept was followed up by morphological studies combined with biochemical analysis, providing compelling evidence that autophagy represents an evolutionarily conserved catabolic machinery in eukaryotic cells for

the degradation of proteins, lipids, and organelles, producing monomers and energy in periods of starvation. The discovery of autophagy-related (ATG) genes that initiate and execute autophagy laid the foundation to dissect the process of autophagy in molecular detail and, moreover, provided the basis to realize that autophagy is intrinsic to cellular survival [1].

The term apoptosis was introduced by John Kerry, Andrew Wyllie, and Alastair Currie in 1972, based on the Greek words *apo* for off and *ptosis* for fall, hence falling off, referring to the leaves falling off the tree. The term was introduced to define a discrete cell death morphology including cell shrinkage, fragmentation, and blebbing, dissimilar to the process of necrosis characterized by cell swelling and lysis, subsequently provoking an inflammatory response. Studies in *Caenorhabditis elegans* (*C. elegans*) laid the foundation to discover the molecular basis of apoptotic cell death, ultimately leading to the current general knowledge that apoptosis is a genetic program of cell death [2].

Although both evolutionarily conserved genetic programs autophagy and apoptosis result in opposing consequences, cellular survival or death, respectively, both are intrinsic to the development, differentiation, and health of eukaryotic organisms, critically secure genomic stability, and fight the onset of age-related human diseases such as cancer [3, 4]. Deduced from this, autophagy and apoptosis should, despite representing distinct genetic programs, be interconnected by conserved context-dependent control mechanisms. In fact, this hypothesis has attracted an enormous amount of interest over the last decade or more, resulting in a great quantity of publications available on this topic. Generally accepted now is the consideration that: i) autophagy inhibits apoptosis and ii) apoptosis inhibits autophagy [5]. However, under certain circumstances autophagy, in particular forms of noncanonical autophagy, contributes to cell death [6]. Moreover, it has been recognized that cell death can also occur by excessive autophagy, referred to as autophagic cell death (ACD). In this context, it is considered that autophagy specifically restricts ACD but that superabundant autophagic degradation mediates ACD [5].

In this chapter, we focus on canonical WD repeat protein interacting with phosphoinositides (WIPI)-mediated autophagy [7] and its regulation through common regulatory factors for both autophagy and apoptosis.

2. The process of WIPI-mediated autophagy

The regulatory relationship between autophagy and apoptosis converges in controlling phosphatidylinositol 3-phosphate (PI3P) production, which initiates autophagosome formation through the PI3P-effector function of WIPI proteins at the onset of autophagy (Figure 1). PI3P production is initiated to induce autophagy upon nutrient or energy deprivation [1]. Low energy levels in the cell activate the AMP-activated kinase (AMPK), which phosphorylates and thereby activates the serine/threonine-specific protein kinase ULK1 (UNC51-like kinase 1) functioning in a principal regulatory complex together with FIP200 (focal adhesion kinase family interacting protein of 200 kD) [8]. AMPK is further controlled by the Ca^{2+}/calmodulin-dependent protein kinase β (CaMKKβ) [9] and liver kinase B1 (LKB1) [10]. However, amino acid deprivation has also been shown to initiate WIPI-mediated autophagy in the absence of AMPK [11].

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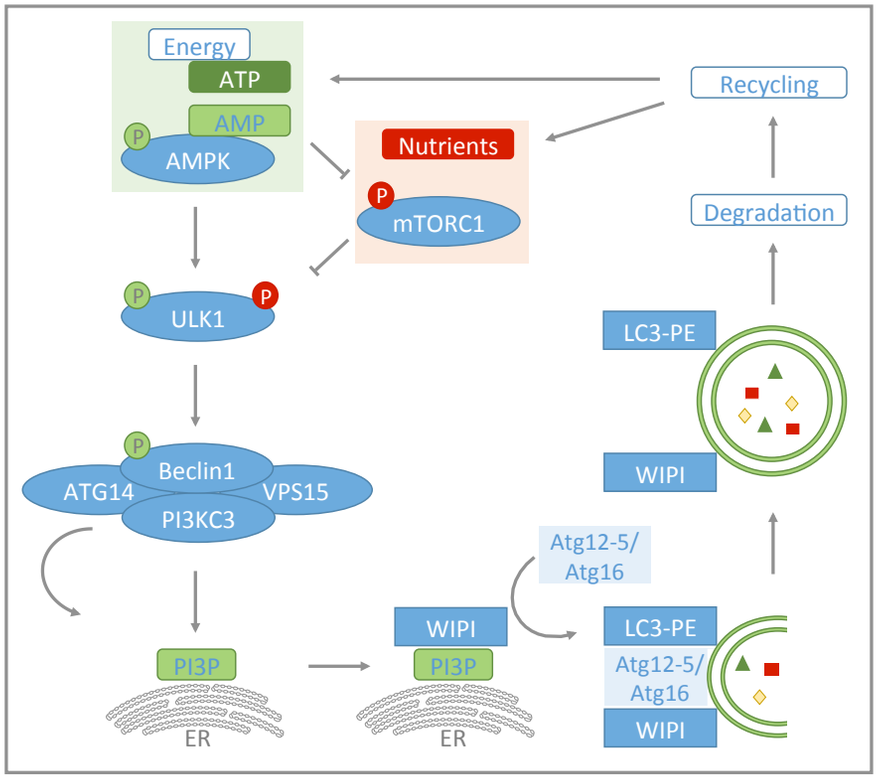

Figure 1. A current working model of WIPI-mediated autophagy

Active ULK1, in complex with FIP200, was shown to phosphorylate Beclin 1, a regulatory factor that in turn forms a complex with the phosphatidylinositol 3-kinase class 3 (PI3KC3), vacuolar protein sorting-associated protein 15 (VPS15) and autophagy-related protein 14 (ATG14) [12]. This core PI3KC3 complex localizes through ATG14 to the endoplasmic reticulum and produces PI3P essential for the formation of initial autophagosomal membranes referred to as phagophore or isolation membrane [13]. The PI3P signal is decoded by WIPI proteins, members of the PROPPIN (beta-propellers that bind phosphoinositides) family with the four human members WIPI1 to WIPI4 considered to function as essential PI3P effectors at the nascent autophagosome (Figure 1) [7, 14].

The identification of human WIPI members was based on initial screening of a human liver cDNA library for novel inhibitors of p53 and it was shown that WIPI genes are ubiquitously expressed in normal human tissue but aberrantly in a variety of human cancer types [14]. By structural homology modeling, it was found that WIPI proteins fold into seven-bladed beta-propellers with an open "Velcro" arrangement harboring a phosphoinositide-binding site specific for the PROPPIN family [7, 14]. Initial phylogenetic analysis revealed that the PROPPIN family consists of two paralogous groups harboring WIPI1/WIPI2 and WIPI3/4 in each of the groups, respectively [14].

WIPI1 is the founding member of the human PROPPIN group [7, 14] currently with the two recognized splice variants WIPI1α [7] and WIPI1β (= WIPI49) [15] WIPI1 plays an important role in autophagy [16] due to the specific binding to PI3P [17]. Upon autophagy induction, WIPI1 localizes to the endoplasmic reticulum, and is then found on the phagophore, and the

autophagosome (Figure 1) [18]. Due to this specific localization, quantitative assessment of PI3P-binding dependent association of WIPI1 with autophagosomal membranes has been established to monitor autophagy in mammalian cells using automated high-throughput imaging [19].

WIPI2 also binds to the phagophore and to autophagosomes due to its specific binding to PI3P [7,18,20]. By comparing four of the many existing WIPI2 splice variants [14], it became apparent that only WIPI2B and WIPI2D, both expressed upon exon skipping, are involved in autophagy but that full-length WIPI2A and also WIPI2C do not seem to respond with an increase in autophagosomal membrane localization upon amino acid starvation [16]. Importantly, WIPI2B was recently shown to specifically recruit components of the autophagy-specific ubiquitin-like conjugation system to the phagophore [21]. Hereby, WIPI2-dependent recruitment of the ATG12-5/ATG16 complex permits the conjugation of LC3 to phosphatidylethanolamine, a process often referred to as LC3 lipidation (Figure 1) [21]. PE-conjugated LC3 is subsequently engaged in phagophore expansion and specific cargo recruitment. LC3 as well as WIPI1 and WIPI2 become membrane proteins of autophagosomes (Figure 1).

So far, PI3P binding as well as autophagosomal membrane localization has not been demonstrated for WIPI3. However, WIPI4 is considered to also bind to the phagophore but downstream of WIPI1 and WIPI2 [7, 22]. Due to the identification of novel *de novo* mutations in WIPI4, loss of WIPI4 function has been linked to SENDA (static encephalopathy of childhood), a sporadic form of NBIA (neurodegeneration with brain iron accumulation) [23]. Based on this finding and the important roles of WIPI proteins in executing autophagy in general, it is predicted that WIPI malfunctions may be intrinsic to a great variety of human pathologies with irregular autophagy [7].

An important evolutionarily conserved inhibitor of autophagy is mTOR (mammalian target of rapamycin). mTOR is a serine-threonine-specific protein kinase and component of two multiprotein complexes, mTORC1 (mTOR complex 1) and mTORC2 (mTOR complex 2) [24]. mTORC1 consists of RAPTOR (regulatory-associated protein of mTOR), PRAS (40kDa Pro-rich Akt substrate), mLST8 (mammalian lethal with SEC13 protein 8), and DEPTOR (DEP domain containing mTOR interacting protein). mTORC1 represents a nutrient sensor in eukaryotic cells and, in its activated form, mTORC1 localizes at the lysosomal surface and receives free amino acids released from the lysosome [24]. mTORC1 can also be activated by a great variety of signaling cascades, most prominently by the insulin/IGF (insulin-like growth factor) receptor pathway via AKT [24]. Importantly, amino acid availability activates mTORC1 and, in consequence, inhibits autophagy. The inhibition of autophagy by mTORC1 is mediated through ULK1 phosphorylation (Figure 1). In this situation, mTORC1 also phosphorylates S6K1 (ribosomal S6-kinase 1) and 4E-BP (eIF4E binding protein), which enhances autophagy-opposing effects, mRNA translation, and protein synthesis [24].

3. Signals that regulate both apoptosis and WIPI-mediated autophagy

Autophagy and apoptosis are co-regulated by a complex signaling crosstalk, generally leading to the inhibition of apoptosis when autophagy is active and the inhibition of autophagy to permit apoptosis (Figure 2).

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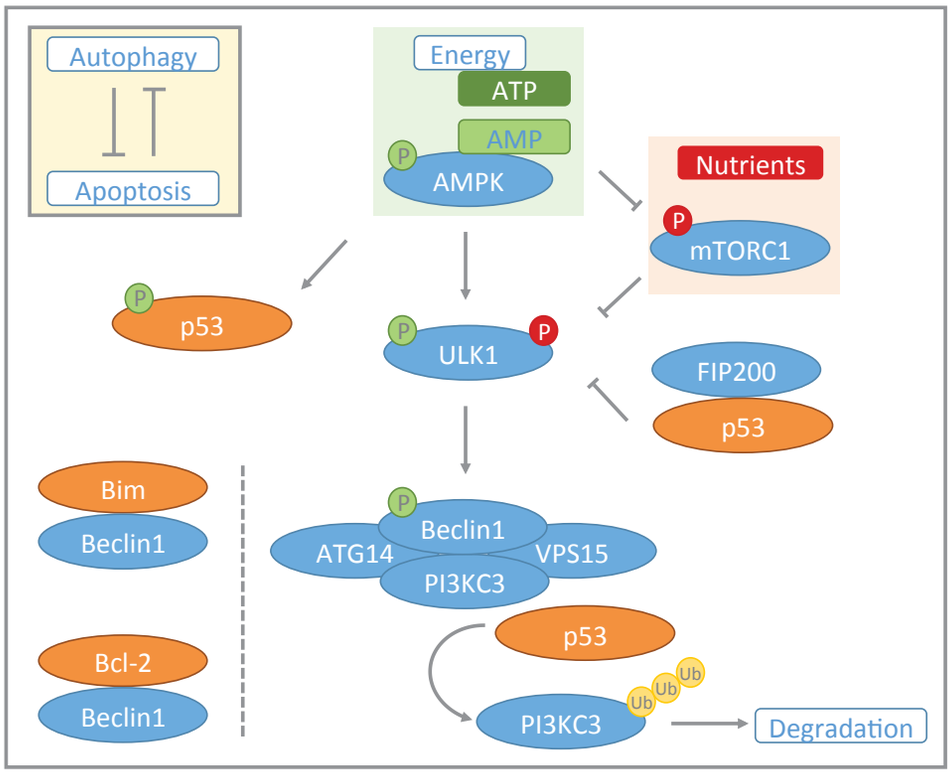

Figure 2. Regulation of autophagy by factors that also regulate apoptosis

Autophagy negatively regulates apoptosis by the specific degradation of apoptotic components including active caspase-8 [25] and the proto-oncogenic c-src (cellular src; src for sarcoma) tyrosine kinase that can initiate apoptosis by activating pro-caspase-9 [26]. Thereby, active c-src is recruited to the phagophore through CBL (casitas B-lineage lymphoma)-mediated binding to LC3 [26]. Subsequently, c-src is degraded through autophagy.

Another apoptosis-preventing result of autophagic activity is the specific removal of damaged mitochondria, termed mitophagy [27]. Depolarization of the mitochondrial membrane indicates a loss of function in the respiratory chain and leads to an accumulation of PINK1 (PTEN-induced putative kinase 1) on the outer mitochondrial membrane. PINK1 recruits and activates the E3 ubiquitin ligase PARKIN that ubiquitinates VDAC1 (Voltage-dependent anion-selective channel protein 1) and MFN1/2 (Mitofusin-1/2). Ubiquitinated VDAC1 and MFN1/2 are recognized by p62 that connects the damaged organelle via LC3 binding to the phagophore [27]. Hence, intrinsic activation of apoptosis due to defective mitochondria is counteracted by autophagy.

A crucial co-regulator of both autophagy and apoptosis is AMPK, which initiates autophagy through ULK1 phosphorylation (Figure 2). Interestingly, active AMPK phosphorylates p53 at serine 15 upon glucose starvation, which activates the transcriptional transactivator activity of p53 to promote an AMPK-dependent cell-cycle arrest at the G1/S boundary [28]. As a consequence, reentering the cell cycle is restricted to glucose availability, securing that dividing cells have enough nutrients available for energy and macromolecule production [28].

On the other hand, p53 inhibits autophagy in several distinct ways (Figure 2). In exhibiting its nonnuclear function, cytoplasmic p53 competes with ULK1 for binding to FIP200 under certain circumstances [29]. As the ULK1/FIP200 association is required for the activation of PI3KC3 and subsequent PI3P production followed by WIPI-mediated autophagy (Figure 1), cytoplasmic p53 inhibits autophagy. This scenario represents an interesting connection to the induction of apoptosis, which can be initiated through the association of cytoplasmic p53 to mitochondria [30].

In general, the site-specific transcriptional transactivator factor activity of p53 in the nucleus is induced upon DNA damage and p53 target genes subsequently permit DNA repair or apoptosis to secure genomic stability. Among the p53 target genes, FBXL20 (F-box and leucine-rich repeat protein 20), known to mediate ubiquitination and proteasome-mediated degradation, is expressed and targets PI3KC3 [31]. Hence, activation of the nuclear function of p53 can also lead to the inhibition of autophagy through p53-promoted PI3KC3 degradation (Figure 2).

Further, common factors regulating autophagy and apoptosis include Bcl-2 (B-cell lymphoma 2) with antiapoptotic function and Bim (Bcl-2-interacting mediator of cell death), a BH3 (Bcl-2 homology domain 2)-only protein with pro-apoptotic function (Figure 2). Bim is bound to the dynein motor protein DYNLL1/LC8 (dynein light chain 1) at microtubules. In nutrient-rich conditions, Bim binds to Beclin 1, preventing Beclin 1 binding to complex with PI3KC3 and initiate WIPI-mediated autophagy. Bim, therefore, inhibits autophagy through Beclin 1 mislocalization to the dynein motor protein [32]. In starvation conditions, Bim releases Beclin 1 and allows Beclin 1/PI3KC3 association and subsequently the induction of autophagy [32].

Bcl-2, a well-known interacting partner of Beclin 1, also prevents Beclin 1 from binding to PI3KC3. The Bcl-2/Beclin 1 association is inhibited upon starvation, leading to JNK (c-Jun N-terminal kinase)-mediated Bcl-2 phosphorylation at multiple sites subsequently releasing Beclin 1 and permitting autophagy activation (Figure 2) [33].

The complex relationship between apoptosis and autophagy regulation is further highlighted by the notion that autophagy-related proteins fulfill pro-apoptotic functions under certain circumstances. During apoptosis, active caspases target AMBRA1, a component of the PI3KC3 complex, for degradation and hence prevent WIPI-mediated autophagy upon the apoptotic point-of-no-return route to cell death [34]. Further, it was demonstrated that calpain 1 and calpain 2, classified as cysteine proteases [35], cleave ATG5 at threonine 193 generating an 24 kDa N-terminal fragment of ATG5 [36]. This ATG5 fragment subsequently translocates to the mitochondrial membrane and initiates cytochrome c release, ultimately promoting the cleavage of pro-caspase-3 into its active form. The effect of fragmented ATG5 is inhibited by high Bcl-2 levels [36]. Moreover, ATG12, the conjugation partner of ATG5, was shown to interact with Bcl-2 via an amino acid sequence in ATG12 resembling a BH3 domain generally known to bind Bcl-2. By binding to Bcl-2, ATG12 inhibits the antiapoptotic function of Bcl-2, leading to an increase of apoptotic cell death [37].

To provide a final example for the complex regulatory crosstalk between apoptosis and WIPI-mediated autophagy, the function of DAPK (death-associated protein kinase 1), a Ca^{2+}/

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calmodulin-sensitive serine/threonine kinase involved in the induction of apoptosis [38], is highlighted in the following section. DAPK phosphorylates Beclin 1 or PKD (protein kinase D) under certain conditions. When reactive oxygen species activate DAPK, DAPK phosphorylates PKD, resulting in the phosphorylation of PI3KC3 by PDK. In turn, PI3KC3 produces PI3P and initiates autophagy [39]. As mentioned, DAPK can also phosphorylate Beclin 1, which occurs at threonine 119 in the BH3-like domain of Beclin 1 responsible for binding to Bcl-2. As Bcl-2-bound Beclin 1 inhibits autophagy, threonine 119 phosphorylation releases Beclin 1 from Bcl2 and enables Beclin1 to bind to PI3KC3 and to stimulate autophagy [40].

4. Outlook

In general, apoptosis and autophagy represent mutually exclusive genetic programs co-regulated by key factors with opposing roles in both pathways. This crosstalk contributes to the survival of healthy cells and secures genomic stability more than one of the pathways alone. Interestingly, key regulatory factors for both apoptosis and autophagy converge on the regulation of PI3P production. WIPI proteins function as essential PI3P effectors at the onset of autophagy; hence, PI3P-dependent localization of WIPI proteins at autophagosomal membranes should reflect both induction of autophagy and inhibition of apoptosis.

The genetic program of apoptosis is executed when the point-of-no-return is reached. In contrast, autophagy was shown to be initiated by the same stimuli that are additionally capable of blocking final autophagic cargo destruction in the lysosomal compartment, or even superstimulate autophagic degradation leading to autophagosomal cell death. Hence, the autophagic pathway not only represents a new rational therapeutic target mechanism for future treatment of human pathologies but also a target mechanism with the benefit to be modulated in many different ways according to the context-dependent needs.

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