WIPI β-propellers in autophagy-related diseases and longevity

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Abstract
Autophagy is a catabolic pathway in which the cell sequesters cytoplasmic material, including long-lived proteins, lipids and organelles, in specialized double-membrane vesicles, called autophagosomes. Subsequently, autophagosomes communicate with the lysosomal compartment and acquire acidic hydrolases for final cargo degradation. This process of partial self-eating secures the survival of eukaryotic cells during starvation periods and is critically regulated by mTORC1 (mammalian target of rapamycin complex 1). Under nutrient-poor conditions, inhibited mTORC1 permits localized PtdIns(3)P production at particular membranes that contribute to autophagosome formation. Members of the human WIPI (WD-repeat protein interacting with phosphoinositides) family fulfil an essential role as PtdIns(3)P effectors at the initiation step of autophagosome formation. In the present article, we discuss the role of human WIPIs in autophagy, and the identification of evolutionarily conserved amino acids of WIPI-1 that confer PtdIns(3)P binding downstream of mTORC1 inhibition. We also discuss the PtdIns(3)P effector function of WIPIs in the context of longevity and autophagy-related human diseases, such as cancer and neurodegeneration.

Introduction
Autophagy secures cellular homoeostasis and survival. Stochastic autophagy leads to a constant renewal of the cytoplasm, and specific autophagy secures the degradation of protein aggregates, damaged organelles and invaded pathogens. Three forms of cellular autophagy, macroautophagy, microautophagy and chaperone-mediated autophagy (CMA) have been described [1]. To date, the most studied form of autophagy is macroautophagy, considered to represent the major autophagic pathway. In the literature, macroautophagy is generally referred to as autophagy, as it is hereinafter.

A hallmark for autophagy is the formation of autophagosomes, double-membrane vesicles that emerge from template membranes, called isolation membranes or phagophores [2]. Autophagosome formation is tightly regulated by mTORC1 (mammalian target of rapamycin complex 1). Inactive mTORC1 allows the initiation of the autophagic pathway by ULK (unc-51-like kinase) 1 and 2 that are controlled further by AMPK (AMP-activated protein kinase) [3]. Autophagosomes fuse with lysosomes to acquire acidic hydrolases for breaking down the sequestered cargo [1,2]. Interestingly, lysosomal positioning of mTORC1 was demonstrated to also control the fusion between autophagosomes and lysosomes [4]. Hence nutrient-sensing of the mTORC1 circuit globally regulates the process of autophagy, from initiation to final cargo degradation [4].

Under nutrient-poor conditions, inactive mTORC1 allows the initiation of autophagy and PtdIns(3)P is generated by PI3KC3 (class III phosphoinositide 3-kinase) in complex with BECLIN 1, VPS15 (vacuolar protein sorting 15) and ATG14L (autophagy 14-like), the latter directing PI3KC3 to initiation sites for autophagosome formation, such as the ER (endoplasmic reticulum) [5]. Generation of PtdIns(3)P represents a crucial initiation step for autophagosome formation [6]. Upon specific binding to locally produced PtdIns(3)P, the PtdIns(3)P signal is received and decoded by WIPI (WD-repeat protein interacting with phosphoinositides) effectors [6,7]. The WIPI effector activity also depends on Ca2+ as chelation counteracts the specific binding of WIPIs to PtdIns(3)P during autophagy initiation [8]. However, local Ca2+ concentration changes were shown to fulfill opposing roles during the process of autophagy [9].

Depending on the PtdIns(3)P effector function of human WIPIs, the two ubiquitin-like conjugation systems specific for autophagy conjugate LC3 (light chain 3) to phosphorylated ethanolamine at the phagophore membrane [10–12]. Subsequently, the phagophore is elongated to form the autophagosome. Membrane rearrangements that occur from autophagy initiation upon local PtdIns(3)P production to the closure of the autophagosomal vesicle are not yet fully understood.

Key words: autophagy, lysosome, mammalian target of rapamycin complex 1 (mTORC1), phosphoinositide 3-phosphate, WD-repeat protein interacting with phosphoinositides (WIPI).

Abbreviations used: DAC-2, abnormal dauer formation 2; ER, endoplasmic reticulum; MITF-M, melanocyte-specific microphthalmia-associated transcription factor isoform; mTORC1, mammalian target of rapamycin complex 1; NBAD, neurodegeneration with brain iron accumulation; PI3KC3, class III phosphoinositide 3-kinase; TOR, target of rapamycin; ULK, unc-51-like kinase; WIPI, WD-repeat protein interacting with phosphoinositides.

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WIPI β-propellers and autophagy
Previously, we identified human WIPI-1 by screening for novel p53-inhibitory factors and subsequently cloned the
our initial phylogenetic analysis revealed that human WIPIs harbour 24 evolutionarily highly conserved amino acids that cluster at two opposite sites of the β-propeller family [19]. The closest WIPI homologues in yeast include Atg18 (functioning in autophagy), Atg21 (functioning in the yeast-specific cytoplasm-to-vacuole pathway) and Hsv2 (functioning in micronucleophagy) [18]. The general term PROPPINs (β-propellers that bind polyphosphoinositides) has also been introduced to include all members of this β-propeller family [19].

According to the ancestral yeast Atg18 function [20], both WIPI-1 and WIPI-2 function as essential PtdIns(3)P effectors during phagophore formation, where WIPI-4 interacts with Atg2 [21], a feature also assigned to yeast Atg18 [22]. On the basis of this, autophagosomal functions originally described for yeast Atg18 should find equivalence in at least three of the human WIPIs (WIPI-1, WIPI-2 and WIPI-4). Of note, the role for WIPI-3 in autophagy has not yet been characterized.

On the basis of functional and immunoelectron microscopy, evidence was provided that WIPIs fulfill essential roles as phosphoinositide effectors throughout the process of autophagy. Upon nutrient starvation, WIPI-1 and WIPI-2 have been found to accumulate rapidly at initiation sites for phagophore formation, the ER and the plasma membrane [10, 23]; WIPI-2 was further detected at membranes close to the Golgi [23]. Moreover, WIPI-1 was detected at phagophore membranes [15], and both WIPI-1 and WIPI-2 were identified as membrane proteins of generated autophagosomes, positioned at the inner and the outer membrane [23]. On the basis of these data, WIPIs should function as PtdIns(3)P effectors from phagophore formation to autophagosome maturation, and may be further involved in the fusion with lysosomes. Indeed, vesicles positive for both WIPI-1 and LAMP-1 (lysosome-associated membrane protein 1) have also been recognized [23]. As WIPIs also bind, to a lesser extent, to further phosphoinositides, in particular PtdIns(3,5)P2 [10, 12, 14, 15], membrane rearrangements occurring throughout the process of autophagy, such as vesicle fusion, might be regulated through different phosphoinositide-binding specificities of human WIPIs.

### WIPI β-propellers and phosphoinositide binding

Our initial phylogenetic analysis revealed that human WIPIs harbour 24 evolutionarily highly conserved amino acids that cluster at two opposite sites of the β-propeller [13]. One such cluster (cluster 1 in Figure 1) contains homologous amino acids across blades 1–4, suggested to bind evolutionarily conserved regulatory factors [13]. Notably, the opposite side of the β-propeller displayed the other cluster of amino acids...
Phosphoinositide binding of human WIPIs, however, should be orchestrated by evolutionarily conserved protein–protein interactions. Evidence for this prediction provides the identification of Arg110 as a critical amino acid in blade 3 of WIPI-1 that, when mutated to alanine, mediates a gain-of-function membrane-binding property independent of the autophagic status of the cell [12] (Table 1). In line with this, the region of association between *Saccharomyces cerevisiae* Atg18 and Atg2 was identified to occur at the same side of the Atg18 β-propeller [26,27]. Hence PtdIns(3)P-dependent autophagosomal membrane association of WIPIs should be regulated by the concerted actions of interacting factors under the control of mTORC1, as suggested by mTORC1 inhibition or down-regulation [12,15], PI3KC3 down-regulation [28], and by a recent report demonstrating that ULKs signal to WIPIs upon starvation [29]. As inactive mTORC1 permits the generation of PtdIns(3)P by PI3KC3, wortmannin administration or PI3KC3 down-regulation should abrogate the PtdIns(3)P effector activity of WIPIs, which indeed was also demonstrated in earlier studies [13,15,28].

However, another study suggested that WIPIs might also act upstream of mTORC1 during melanogenesis [30]. Melanosomes are lysosome-related organelles in which melanin is synthesized. The master transcriptional regulator of melanosome-specific proteins is the...
melanocyte-specific microphthalmia-associated transcription factor isoform MITF-M. It was found that WIPI-1 depletion decreased the expression of both MITF-M and its target genes [30]. However, the depletion of mTORC1 pathway components or rapamycin treatment activated MITF-M. As the effect of rapamycin could complement the phenotype observed in WIPI-1-depleted cells, it was concluded that WIPI-1 acts upstream of mTORC1 and activates the transcription of melanogenic proteins upon mTORC1 inhibition. However, it was also suggested that mTORC1 and WIPI-1 might regulate melanogenesis in an autophagy-independent manner [30].

**WIPI β-propellers and diseases**

There is increasing evidence that autophagy contributes to the development of a variety of age-related human diseases, such as cancer and neurodegeneration [1]. The contribution of autophagy to human pathologies can result from malfunctions in the autophagy machinery, i.e. mutations in BECLIN 1 (tumorigenesis) or ATG16L (Crohn’s disease) [31]. In contrast, pathologically altered cells specifically up-regulate autophagy to compete with normal cells for cellular survival, as generally predicted for therapy-resistant tumour cells [32].

WIPIs, ubiquitously expressed in normal human tissues with particular high levels in muscle and heart, are aberrantly expressed in a variety of matched human tumour samples [13]. WIPI-1 is encoded within a region on the long arm of chromosome 17 that has been linked to breast and ovarian cancer. Consistent with this, WIPI-1 was differentially expressed in both ovarian [33] and breast tumour samples, and was found further to be down-regulated in kidney tumours (Table 2). Moreover, WIPI-1 expression was prominently up-regulated in skin cancer patients and malignant melanoma-derived human tumour cell lines [13], and it was suggested that WIPI-1 should become employed as a biomarker for melanomas (Table 2). Up-regulation of WIPI-1 in certain cancers was further indicated by analysing an in vitro lung tumour model [34] and by analysing matched human normal/cervical tumour samples [13]. WIPI-2 and WIPI-4 both showed a prominent down-regulation in kidney and pancreatic tumour samples, and WIPI-3 was aberrantly expressed in ovarian, uterine and kidney tumours [13]. These studies suggest that WIPIs contribute to tumorigenesis; however, large-scale analyses with regard to the expression status of human WIPIs during different stages of tumorigenesis are urgently needed.

Apart from cancer, WIPI-1 was also implicated in other human diseases, such as phospholipidosis [35], a lysosomal storage disorder, and potentially also in cardiovascular diseases [36]. Whether or not these features correlate with the autophagic function of WIPI-1 needs to be addressed in the future; however, altered autophagic activities have been generally assigned to these human diseases.

There is strong evidence to suggest that suppressed basal autophagy impairs the clearing of intracellular protein aggregates, ultimately leading to a variety of neurodegenerative diseases. Strikingly, *de novo* mutations in human WIPI-4 have recently been identified and linked to NBIA (neurodegeneration with brain iron accumulation) patients [37,38]. NBIA is characterized by excessive iron accumulation in the brain, leading to symptoms such as dystonia and parkinsonism. The mechanism leading to this type of neurodegeneration remains unknown, but should critically involve the phosphoinositide-effector activity of human WIPI-4 during basal autophagic clearance of protein aggregates.

**WIPI β-propellers and longevity**

Similar to higher eukaryotes, in the nematode *Caenorhabditis elegans*, autophagy has a role in survival during starvation, development and cellular homeostasis. Upon decreased activity of DAF-2 (abnormal dauer formation), the insulin/IGF-1 receptor homologue in *C. elegans*, the nematode’s lifespan doubles in comparison with wild-type animals [39]. In line with this finding, down-regulation of LET-363, the homologue of mTOR (mammalian target of rapamycin) in *C. elegans*, also results in an increased lifespan [40], suggesting that eukaryotic lifespan is regulated by insulin and the TOR (target of rapamycin) signalling circuit. In this context, it was demonstrated that autophagy genes are also necessary for lifespan extension in *C. elegans* [41]. Additionally, a decrease in both food uptake [42] and mitochondrial activity [43] also delayed the aging of the nematode.

In *C. elegans*, ATG-18 and EPG-6 share the highest primary sequence homology with human WIPI-1/2 and WIPI-3/4 respectively [13]. The involvement of ATG-18 in the longevity of the nematode has been investigated. It was shown that both mutation and maternal RNAi (RNA interference) of *atg-18* slightly decreased the lifespan of *C.

### Table 2 | WIPIs and diseases

<table>
<thead>
<tr>
<th>WIPI</th>
<th>Associated disease</th>
<th>Reference(s)</th>
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<tbody>
<tr>
<td>WIPI-1</td>
<td>Skin cancer</td>
<td>[13]</td>
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<td>Cervical cancer</td>
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<td></td>
<td>Kidney cancer</td>
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<tr>
<td>WIPI-1</td>
<td>Ovarian cancer</td>
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<tr>
<td>WIPI-1</td>
<td>Lung cancer</td>
<td>[34]</td>
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<tr>
<td>WIPI-1</td>
<td>Phospholipidosis</td>
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<tr>
<td>WIPI-1</td>
<td>Cardiovascular</td>
<td>[36]</td>
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<tr>
<td>WIPI-1</td>
<td>Skin cancer (melanoma)</td>
<td>[46]</td>
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<tr>
<td>WIPI-1</td>
<td>Breast cancer</td>
<td>[47]</td>
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<tr>
<td>WIPI-2</td>
<td>Pancreatic cancer</td>
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<td>Kidney cancer</td>
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<tr>
<td>WIPI-3</td>
<td>Ovarian cancer</td>
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<td></td>
<td>Uterine cancer</td>
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<td>WIPI-4</td>
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<td>Kidney cancer</td>
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<tr>
<td>WIPI-4</td>
<td>NBIA</td>
<td>[37,38]</td>
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elegans and that wild-type atg-18 is required for the increased lifespan of mutant daf-2 animals [44,45]. The role of ATG-18 in aging was also represented by an early mortality decline in atg-18 mutant nematodes, and the fact that wild-type atg-18 is necessary for lifespan extension upon both LET-363 down-regulation and decreased mitochondrial activity [44,45].

As the increased lifespan of nematodes with reduced insulin and TOR signalling, dietary restriction and decreased mitochondrial activity is counteracted by mutant or depleted autophagy-related gene products, including the WIPI-1/2 homologue atg-18, it is assumed that lifespan regulation in eukaryotic organisms in general converge on autophagy genes [44].

Conclusions and future directions

We suggest that the autophagic function of human WIPI-β-propellers is defined by evolutionarily highly conserved amino acids that confer specific PtdIns(3)P binding on one side of the β-propeller, and multiple regulatory protein–protein interactions on the opposite propeller side (Table 1 and Figure 1). The essential role of human WIPIs in a variety of human age-related diseases should be addressed in the future, as both WIPI mutations as well as altered regulation of human WIPIs are predicted to critically contribute to many pathological phenotypes. As the PtdIns(3)P effector activity of human WIPIs is regulated by mTORC1, lifespan many pathological phenotypes. As the PtdIns(3)P signal needs to be addressed in future studies.

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