科学通报 2023 年 第 68 卷 第 27 期: 3698 ~ 3706
评 述
《中国科学》杂志社
SCIENCE CHINA PRESS

WIPI2在经典自噬和非经典自噬中的作用

许银丰[1], 万伟[2*]

1. 湖南第一师范学院基础生物学实验室, 长沙 410205;
2. 浙江大学医学院生物化学系, 杭州 310058
* 联系人, E-mail: wanwei@zju.edu.cn

2023-03-22 收稿, 2023-05-06 修回, 2023-05-06 接受, 2023-05-07 网络版发表
国家自然科学基金(31970694)、湖南省自然科学基金(2022JJ30186)、湖南省科技创新计划(2022RC1171)和长沙市杰出创新青年培养计划(kq2206049)资助

摘要 细胞自噬是真核生物特有的依赖溶酶体的细胞内降解途径, 通过清除蛋白聚集体、受损细胞器和入侵病原体等细胞内容物, 维持细胞内环境稳定. WIPI蛋白是在哺乳动物细胞中鉴定的与细胞自噬密切相关的一类蛋白家族. 其中, WIPI2主要调控自噬前体的延伸过程, 是直接参与自噬小体形成的少数必需蛋白之一. WIPI2可通过多种不同的作用方式行使功能. 近年来的研究发现, 除了营养剥夺诱导的经典自噬, WIPI2对于病毒感染诱导的非经典自噬同样必不可少. 而且, 在不同类型的自噬途径中, 不同的细胞内外刺激信号可通过调控WIPI2的蛋白稳定性或者WIPI2与自噬前体的亲和力, 影响细胞内的自噬水平, 帮助细胞更好地应对内外环境的改变. 值得一提的是, WIPI2的功能缺失突变与一类引起发育障碍的人类遗传病相关. 本文主要围绕WIPI2在经典自噬和非经典自噬中的作用方式与调控机制, 综述近年来的研究进展.

关键词 WIPI2, 经典自噬, 非经典自噬, 自噬小体, 自噬前体

WIPI2(WD repeat domain phosphoinositide-interacting protein 2)蛋白属于哺乳动物细胞中WIPI蛋白家族成员, 其在酵母中的同源蛋白为Atg18(autophagy-related protein 18)和Atg21[1~4]. 所有WIPI蛋白家族成员中, WIPI2是细胞自噬(autophagy)过程中发挥主要调控作用的分子[3]. 近年来的研究表明, 在营养剥夺等诱导的经典自噬(canonical autophagy)和病毒感染等诱导的非经典自噬(non-canonical autophagy)中, WIPI2均为不可或缺的关键蛋白. 在自噬前体(phagophore)延伸形成双层膜结构的自噬小体(autophagosome)过程中, WIPI2属于为数不多的几个直接参与其中的核心组分之一[5,6]. 随着自噬相关研究的不断深入, 借助于蛋白质组学和结构生物学等先进研究技术, WIPI2在细胞自噬中多种不同的作用方式被陆续发现. 此外, 越来越多的细胞内外刺激信号也被发现, 通过影响WIPI2, 调控不同的自噬类型中自噬小体的形成过程[7~9]. 近年来, WIPI2与人类疾病发生发展的关系也逐渐得到越来越多的关注和研究. 本文旨在综述和讨论WIPI2在经典自噬和非经典自噬中的不同作用方式, 作用于WIPI2的各种细胞自噬调控机制, 以及WIPI2与人类疾病发生发展的潜在联系, 以期为经典自噬和非经典自噬相关的研究提供借鉴和参考.

1 WIPI蛋白家族

在哺乳动物细胞中, WIPI蛋白家族含有4个成员, 包括WIPI1、WIPI2、WIPI3和WIPI4. 基于氨基酸序列保守性的分子进化分析显示, 哺乳动物细胞中的WIPI蛋白家族与酵母中的Atg18和Atg21具有共同的起源.

引用格式: 许银丰, 万伟. WIPI2在经典自噬和非经典自噬中的作用. 科学通报, 2023, 68: 3698–3706
Xu Y F, Wan W. Emerging roles of WIPI2 in canonical and non-canonical autophagy (in Chinese). Chin Sci Bull, 2023, 68: 3698–3706, doi: 10.1360/TB-2023-0263

相对而言, WIPI1和WIPI2与酵母中Atg18的同源性高于Atg21. 而在WIPI蛋白家族的4个成员之间, WIPI1与WIPI2的同源性更高, WIPI3则与WIPI4的同源性更高(图1)[3]. WIPI蛋白家族均含有保守的结合脂质3-磷酸磷脂酰肌醇(phosphatidylinositol-3-phosphate, PI3P)的氨基酸基序, 负责将WIPI蛋白家族靶向富含PI3P的自噬前体处发挥功能[3,10].

WIPI蛋白家族中, WIPI2是在自噬前体的延伸过程中发挥主导作用的成员. 在细胞内敲低*WIPI1*, 自噬小体的形成和自噬底物的降解均不会受到明显的影响[3], 表明WIPI1很可能不直接参与细胞自噬的调控. 有趣的是, WIPI1在黑色素瘤和骨肉瘤等不同癌症中显著高表达[11,12]. 初步的机制研究揭示, WIPI1通过抑制CDKN1A、CDK4和CCND1等细胞周期相关调控因子的表达, 促进细胞周期过程G_1/S期的转换, 从而促进细胞增殖[12]. 小鼠荷瘤实验显示, 在肿瘤细胞内敲低*WIPI1*, 显著降低了肿瘤的大小和重量[12]. 然而, WIPI1调控基因转录的机制尚不明确, 其是否需要通过结合PI3P发挥功能, 也有待研究. 与WIPI1和WIPI2不同, WIPI3和WIPI4可作为支架蛋白参与AMPK(AMP-activated protein kinase)信号通路的转导, 在营养剥夺诱导的经典自噬启动过程中发挥一定的调控作用[13]. 此外, 近年来的多项研究发现, 自噬前体处的WIPI3和WIPI4通过与介导脂质转运的ATG2A/B相互作用, 将内质网处的多种脂质特异性地转运至自噬前体处, 促进自噬前体的延伸和自噬小体的形成[14~17].

2 自噬小体形成的基本过程

细胞自噬是真核细胞内依赖溶酶体(lysosome)的降解途径, 其标志事件是在细胞内形成双层膜结构的自噬小体. 在诱导自噬的条件下, 自噬前体延伸形成闭合的自噬小体, 与此同时将细胞质中的蛋白聚集体、受损细胞器和入侵病原体等自噬底物包裹其中. 此后, 成熟的自噬小体与溶酶体融合, 并利用溶酶体来源的各类水解酶降解自噬底物, 维持细胞内环境稳定[18,19].

在营养剥夺、氧气不足或者热激等刺激条件下, 细胞内的能量感受器蛋白激酶AMPK激活或者营养感受器蛋白激酶mTORC1(mechanistic target of rapamycin complex 1)失活, 继而引发蛋白激酶ULK1(unc-51-like kinase 1)复合物的激活. ULK1复合物通过磷酸化脂激酶VPS34/PIK3C3(phosphatidylinositol 3-kinase catalytic subunit type 3)复合物的多个亚基提高VPS34复合物的

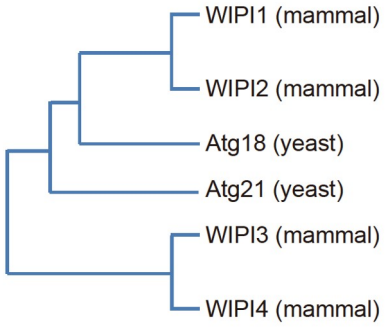

图 1 (网络版彩色)哺乳动物细胞中的WIPI蛋白家族是酵母中Atg18和Atg21的同源蛋白. 哺乳动物细胞中的WIPI1和WIPI2与酵母中的Atg18亲缘关系更近, 哺乳动物细胞中的WIPI3和WIPI4则与酵母中的Atg21亲缘关系更近

Figure 1 (Color online) Mammalian WIPI-family proteins are the orthologues of yeast Atg18 and Atg21. WIPI1 and WIPI2 in mammalian cells are closely related to Atg18 in yeast, and WIPI3 and WIPI4 in mammalian cells are closely related to Atg21 in yeast

活性, 促使后者合成脂质PI3P, 启动自噬小体的形成(图2)[20~22].

ATG8蛋白家族, 包括LC3(microtubule-associated protein 1 light chain 3)和GABARAP(gamma-aminobutyric acid receptor-associated protein)蛋白家族, 在自噬前体延伸形成自噬小体的过程中发挥关键作用. 全长的ATG8蛋白家族在半胱氨酸蛋白酶ATG4的切割下暴露出羧基端的甘氨酸残基, 然后在两个类泛素化系统(ubiquitination-like conjugation system)的协助下, 细胞质中水溶性的ATG8蛋白家族羧基端的甘氨酸残基共价连接上脂质磷脂酰乙醇胺(phosphatidyl ethanolamine, PE), 进而转化为自噬前体膜结合的ATG8-PE. 在ATG12系统中, 在E1样酶ATG7和E2样酶ATG10的协助下, ATG5共价连接到ATG12上, ATG5-ATG12继而通过蛋白相互作用与ATG16L1组装成为E3样酶ATG16L1-ATG5-ATG12复合物. 在ATG8系统中, ATG8蛋白家族在E1样酶ATG7、E2样酶ATG3和E3样酶ATG16L1-ATG5-ATG12复合物的协助下, 共价连接到ATG8蛋白家族上形成ATG8-PE. 需要注意的是, ATG8-PE也可在ATG4的切割下重新释放出游离的水溶性的ATG8蛋白家族.

自噬前体膜结合的ATG8-PE通过招募下游的多种ATG8结合蛋白参与调控细胞自噬的多个阶段, 包括自噬小体的形成、自噬底物的招募和自噬溶酶体的形成等[23]. ATG8蛋白家族主要通过两种方式招募下游蛋白. 很多下游蛋白含有一个或者多个保守的ATG8结合基

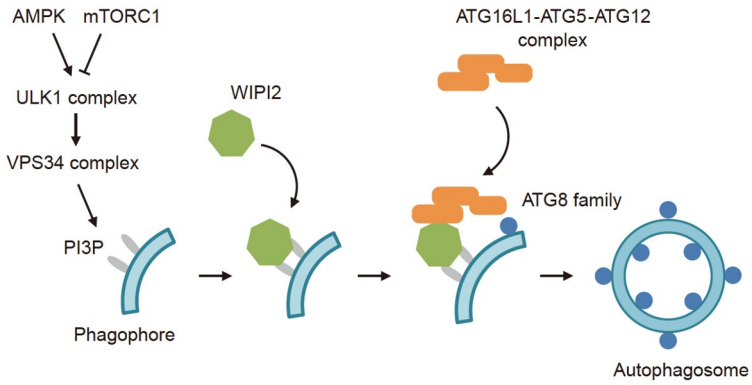

图 2 (网络版彩色)自噬小体形成的基本过程. AMPK激活或者mTORC1失活等上游自噬信号, 激活ULK1复合物, 后者继而激活VPS34复合物, 促进脂质PI3P的合成, 导致细胞自噬的启动. 自噬前体通过膜上的PI3P招募WIPI2, 后者继而通过蛋白相互作用招募ATG16L1-ATG5-ATG12复合物, 促进ATG8蛋白家族在自噬前体膜上完成最终的脂质化修饰. ATG8蛋白家族随之由水溶性的蛋白最终转化为膜结合形成, 膜结合的ATG8蛋白家族通过募集下游的多种不同的效应蛋白促进自噬前体延伸形成自噬小体

Figure 2 (Color online) An overview of autophagosome formation. Stimulated by upstream autophagic signaling, such as AMPK activation or mTORC1 inactivation, ULK1 complex is activated and leads to the activation of VPS34 complex, which triggers PI3P synthesis and subsequent autophagy induction. Phagophore-localized PI3P binds to WIPI2, which mediates the recruitment of ATG16L1-ATG5-ATG12 complex for the lipidation of ATG8-family proteins and leads to the conversion of soluble ATG8-family proteins into membrane-bound forms. Membrane-bound ATG8-family proteins then recruit a number of downstream effectors to promote phagophore expansion and subsequent autophagosome formation

序(ATG8-interacting motif, AIM)或者泛素结合基序(ubiquitin-interacting motif, UIM), 它们可通过AIM或者UIM与ATG8蛋白家族相互作用, 进而被募集至ATG8-PE所在的自噬膜处[24,25].

上游PI3P信号通过何种机制衔接下游ATG8蛋白家族的脂质化过程, 曾经是细胞自噬领域长期悬而未决的重大科学问题. WIPI2的发现完美地解决了这一问题. WIPI2作为桥梁分子, 一方面结合PI3P, 一方面通过与ATG16L1相互作用招募ATG16L1-ATG5-ATG12复合物, 从而促进ATG8蛋白家族在富含PI3P的自噬前体处完成脂质化修饰(图2)[3,5].

3 WIPI2在自噬小体形成中的作用

3.1 WIPI2在非选择性自噬中的作用

依据自噬底物的不同, 细胞自噬可分为非选择性自噬(nonselective autophagy)和选择性自噬(selective autophagy)[26,27]. 营养剥夺诱导的细胞自噬即属于最广为研究的非选择性自噬. WIPI2在细胞自噬中的调控作用最早也是基于营养剥夺诱导的非选择性自噬阐明的[3].

最早的研究发现, WIPI2可作为PI3P的效应蛋白招募ATG16L1-ATG5-ATG12复合物, 促进自噬前体的延伸(图3)[3,5]. 蛋白结构研究不仅解析了WIPI2和ATG16L1特异性相互作用的分子基础, 而且确定了介导二者相互作用的关键氨基酸位点[28,29]. 有趣的是, 蛋白结构的研究还发现了ATG16L1上新结合WIPI2的氨基酸位点, 且该位点从酵母到哺乳动物均高度保守[28], 表明WIPI2和ATG16L1的结合并非哺乳动物细胞特有的自噬调控模式. 除了PI3P, 脂质5-磷酸磷脂酰肌醇(phosphatidylinositol-5-phosphate, PI5P)也可通过直接的结合作用介导WIPI2的招募, 在葡萄糖缺乏诱导的细胞自噬中发挥作用(图3)[30]. 此外, 多项研究发现, 脂质4-磷酸磷脂酰肌醇(phosphatidylinositol-4-phosphate, PI4P)和脂质双磷酸化的磷脂酰肌醇(phosphatidylinositol 3,5-bisphosphate, PI(3,5)P_2)对于WIPI2或者其他WIPI蛋白, 也表现出较强的亲和力[7,31]. 然而, PI4P和PI(3,5)P_2是否在细胞自噬过程中通过结合WIPI2发挥作用, 仍不清楚. 此外, WIPI蛋白是否通过结合不同的脂质在细胞自噬以外的生物学过程中扮演未知的角色, 也值得深入研究. 除了脂质, 自噬前体处的RAB11A蛋白也可以介导WIPI2的招募(图3). 而且, RAB11A能够显著提高PI3P与WIPI2的结合能力, 进一步促进PI3P对WIPI2的招募[32]. 体外重建LC3蛋白脂质化修饰反应的实验揭示, WIPI2与ATG16L1的结合, 除了介导ATG16L1-ATG5-ATG12复合物的招募, 还能提高ATG16L1-ATG5-ATG12复合物的酶活. 此外, WIPI2结合自噬前体膜处的PI3P后, 还可提高VPS34复合物的膜

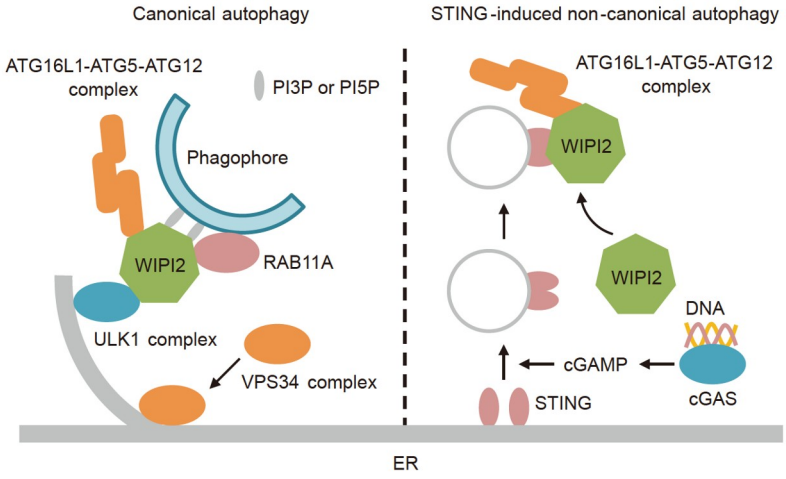

图 3 (网络版彩色)WIPI2在自噬前体延伸过程中的多种作用方式. 在经典自噬中, WIPI2通过多种不同的方式发挥功能. 激活的VPS34复合物催化合成的脂质PI3P以及蛋白RAB11A主要负责招募WIPI2至自噬前体. WIPI2继而通过与ATG16L1相互作用招募ATG16L1-ATG5-ATG12复合物, 促进自噬前体的延伸. WIPI2还可通过与内质网定位的ULK1复合物相互作用, 介导自噬前体与内质网之间形成膜接触, 调控自噬前体的延伸. 在STING介导的自噬中, WIPI2也参与调控自噬前体的延伸过程. 在细胞质DNA的刺激下, cGAS催化合成cGAMP, 后者结合内质网定位的STING, 并促使后者从内质网分离, 形成独立的STING囊泡. STING通过与WIPI2直接相互作用将后者招募至STING囊泡处, 继而介导ATG16L1-ATG5-ATG12复合物的招募, 促进ATG8蛋白家族的脂质化和自噬前体的延伸

Figure 3 (Color online) Multiple roles of WIPI2 in phagophore expansion. During canonical autophagy, several different mechanisms have been demonstrated to regulate phagophore expansion. PI3P, produced by activated VPS34 complex, and RAB11A are responsible for WIPI2 recruitment to the phagophore. After that, WIPI2 recruits ATG16L1-ATG5-ATG12 complex to the phagophore by interacting with ATG16L1, and promotes the lipidation of ATG8-faminly proteins and the expansion of phagophore. In addition, WIPI2 is reported to mediate the membrane contact between phagophore and endoplasmic reticulum (ER) for phagophore expansion through interacting with ER-localized ULK1 complex. During STING-induced autophagy, WIPI2 is also necessary for phagophore expansion. Stimulated by cytoplasmic DNA, activated cGAS synthesizes cGAMP, which binds to ER-localized STING and triggers STING vesicle formation. STING directly interacts with WIPI2 to recruit WIPI2 to STING vesicles. After that, WIPI2 mediates the recruitment of ATG16L1-ATG5-ATG12 complex for the lipidation of ATG8-family proteins, leading to phagophore expansion

结合能力, 促进VPS34复合物依托于膜组分合成PI3P[33]. 以上WIPI2在细胞自噬过程中多种不同的作用方式, 最终均会促进细胞内ATG8蛋白家族的脂质化修饰, 从而调控自噬前体的延伸.

近年的一项研究发现, 自噬前体定位的WIPI2可通过与内质网(endoplasmic reticulum, ER)定位的ULK1复合物相互作用, 介导自噬前体与内质网之间形成膜接触(membrane contact), 进而调控自噬前体的延伸(图3)[34]. 有趣的是, 缺失EGP-3/VMP1造成自噬前体与内质网持续结合, 则会抑制自噬小体的成熟[34]. 以上研究表明, 自噬前体与内质网之间短暂而非持续的膜接触对于自噬前体的延伸是必要的.

3.2 WIPI2在选择性自噬中的作用

选择性自噬可依据自噬底物分为多种不同的类型, 如自噬底物为入侵病原菌的异源自噬(xenophagy)、自噬底物为受损线粒体的线粒体自噬(mitophagy)和自噬底物为破碎内质网的内质网自噬(ER-phagy)等[26,27].

WIPI2在选择性自噬中的功能最早是基于异源自噬发现的. 在沙门氏菌感染细胞诱导的异源自噬中, WIPI2定位的自噬前体膜可以靶向入侵的沙门氏菌[5,9]. 在细胞内敲低*WIPI2*, 则会显著促进细胞内沙门氏菌的增殖[5], 表明WIPI2在沙门氏菌的自噬性清除中发挥作用. 近年的一项研究发现, WIPI2也可参与调控线粒体自噬. 在诱导线粒体自噬的条件下, WIPI2定位的自噬前体膜靶向受损的线粒体, 进而招募VCP(valosin containing protein)蛋白, 促进线粒体外膜蛋白的降解和线粒体自噬的起始[35].

除了异源自噬和线粒体自噬, WIPI2是否在其他类型的选择性自噬中发挥功能仍不甚明确, 有待于未来的研究予以阐明.

3.3 WIPI2在非经典自噬中的作用

无论是营养剥夺诱导的非选择性自噬, 还是入侵病原菌诱导的选择性自噬, 均属于经典自噬的范畴. 研究发现, 多种特定刺激诱导的细胞自噬并不依赖某些关键的自噬基因, 此种类型的细胞自噬被称作非经典自噬[6,36,37].

细胞内的DNA感应通路是机体固有免疫的重要组成部分. 机体组织损伤或者入侵病原体泄漏来源的细胞质DNA结合cGAS(cyclic GMP-AMP synthase)蛋白后, 促进后者合成第二信使cGAMP分子, cGAMP进而与内质网定位的STING(stimulator of interferon genes)蛋白结合, 导致STING从内质网处转运出来形成STING囊泡, 进而招募并激活蛋白激酶TBK1(TANK-binding kinase 1)和下游的转录因子IRF3(interferon regulatory factor 3), 促进干扰素和炎性细胞因子的表达[38,39]. 研究表明, 激活的cGAS-STING通路可通过诱导细胞自噬清除细胞质DNA和入侵的病原体[6,40~45]. 更重要的是, 此种类型的细胞自噬不需要ATG9A、ULK1复合物和VPS34复合物等关键的自噬蛋白与蛋白复合物[6,38].

有趣的是, WIPI2为cGAS-STING通路诱导的非经典自噬所需要[6]. 研究发现, cGAS-STING通路激活以后, 源自内质网的STING囊泡转运至ERGIC(ER-Golgi intermediate compartment)处后充当自噬前体, 利用STING通过蛋白相互作用直接将WIPI2招募至自噬前体处, 促进自噬小体的形成(图3)[42,43]. 值得一提的是, WIPI2上结合STING的关键氨基酸位点也负责结合PI3P. STING和PI3P竞争性结合WIPI2, 导致STING介导的非经典自噬和PI3P介导的经典自噬抑制彼此的发生[42,43].

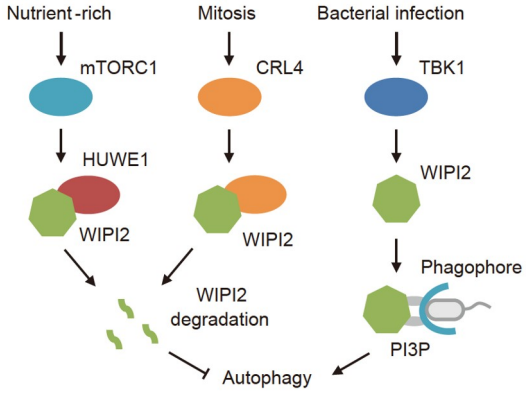

图 4 (网络版彩色)作用于WIPI2的细胞自噬调控方式. 在营养丰富的条件下, 激活的mTORC1直接磷酸化WIPI2并促进其与E3泛素连接酶HUWE1相互作用, 导致WIPI2经由蛋白酶体降解并引起自噬抑制. 在诱导细胞有丝分裂的条件下, 激活的E3泛素连接酶CRL4招募WIPI2并促进后者通过蛋白酶体降解, 同样引起自噬抑制. 在细菌感染条件下, 激活的TBK1在WIPI2定位的自噬前体靶向入侵细菌的过程中发挥重要作用, 其通过诱导异源自噬的起始促进细菌的自噬性清除

Figure 4 (Color online) WIPI2-based mechanisms for autophagy regulation. Under nutrient-rich conditions, activated mTORC1 phosphorylates WIPI2 and promotes its binding to E3 ubiquitin ligase HUWE1, leading to the proteasomal degradation of WIPI2 and the suppression of autophagy. Upon mitosis induction, activated E3 ubiquitin ligase CRL4 interacts with WIPI2 and promotes its proteasomal degradation, resulting in autophagy inhibition. During bacterial infection, TBK1 is activated and promotes the recruitment of WIPI2-localized phagophores to the invading bacteria, leading to the initiation of xenophagy and the clearance of bacteria

4 作用于WIPI2的细胞自噬调控方式

4.1 细胞营养状态对WIPI2的调控作用

细胞的营养状态可通过调控WIPI2的蛋白水平影响细胞自噬的发生强度. 在营养丰富的条件下, 激活的蛋白激酶mTORC1直接磷酸化WIPI2的S395位点, 促进后者与E3泛素连接酶HUWE1(HECT, UBA and WWE domain-containing 1)相互作用, 导致WIPI2发生泛素化并经由蛋白酶体(proteasome)进行降解, 进而降低细胞内WIPI2的蛋白水平并抑制细胞自噬的发生(图4). 而在营养剥夺条件下, mTORC1失活, WIPI2的蛋白水平随之升高并促进自噬小体的形成[7,46]. 显然, 除了调控细胞自噬的启动[47,48], mTORC1还可通过影响细胞内WIPI2的蛋白稳定性调控自噬前体的延伸.

4.2 细胞有丝分裂对WIPI2的调控作用

诱导细胞有丝分裂的条件下, 细胞内的自噬水平显著下降[8,49]. 研究发现, 细胞有丝分裂期间, 细胞内WIPI2的蛋白含量显著下降, 导致细胞自噬受到抑制. 机制研究揭示, 细胞有丝分裂期间, E3泛素连接酶CRL4(CUL4-RING ubiquitin ligases)的活性上调, 导致其底物WIPI2发生泛素化并经由蛋白酶体进行降解(图4)[8]. 然而, 诱导细胞有丝分裂的条件下, 何种信号调控泛素连接酶CRL4的激活仍不明确. 此外, 除了WIPI2, 其他自噬相关蛋白是否也受到CRL4的调控, 值得研究.

4.3 细菌感染对WIPI2的调控作用

在细菌感染诱导异源自噬的条件下, WIPI2定位的自噬前体膜可靶向入侵细菌并促进细菌的自噬性清除[5,9]. 然而, 在异源自噬过程中, WIPI2靶向噬前体膜和自噬前体膜靶向入侵细菌受到细胞内何种信号的调控仍不明确. 研究发现, 丧失结合PI3P能力的WIPI2突变体与入侵细菌在细胞内的共定位消失. 与此一致的是, 抑制细胞内VPS34复合物的活性, 从而降低细胞内的PI3P含量, 也会抑制WIPI2与入侵细菌的共定位[9].

此外, 敲除细胞内的蛋白激酶*TBK1*或者阻断TBK1的激酶活性, 也能显著抑制WIPI2与入侵细菌的共定位[9].

以上研究表明, 细胞内的脂质PI3P和蛋白激酶TBK1在WIPI2靶向噬前体膜或者自噬前体膜靶向入侵细菌过程中发挥重要作用(图4). 然而, PI3P与TBK1在这一过程中具体的功能和潜在的分子机制, 以及二者之间是否存在相互调控关系仍不明确.

5 WIPI2与人类疾病的关系

细胞自噬功能的紊乱与癌症、代谢性疾病和神经退行性疾病等人类重大疾病的发生发展密切相关[50~53]. 研究表明, WIPI2的纯合突变与一类导致发育障碍的人类遗传病相关, 携带该突变的患者表现出智力发育障碍、身形矮小和骨骼发育异常等症状. 机制研究发现, 患者细胞中的WIPI2蛋白249位的缬氨酸突变为甲硫氨酸, 导致WIPI2结合ATG16L1的能力显著下降, 从而引起细胞自噬抑制[54].

考虑到细胞自噬的异常与多种人类疾病相关, 携带突变WIPI2的患者是否对于其他类型的自噬相关疾病表现出更高的易感性, 值得深入研究. 相关的研究发现也将有助于阐明细胞自噬功能的紊乱与人类疾病发生发展的关系.

6 结论与展望

细胞自噬作为真核生物特有的细胞内降解途径, 对于维持细胞内环境稳定至关重要. 随着自噬研究的深入, 多种不同的自噬类型被陆续鉴定. 很多在经典自噬中发挥作用的蛋白并不为某些特定条件诱导的非经典自噬所需要. 然而, WIPI2在经典自噬和非经典自噬中均参与调控自噬前体的延伸过程, 是少数在不同自噬类型中均不可或缺的核心成员之一. 目前, 多种作用于WIPI2的信号通路也被发现在不同的自噬类型中发挥关键的调控作用. WIPI2在细胞自噬中的作用逐渐得到越来越多的关注和研究.

WIPI蛋白家族各个成员在细胞自噬中的作用, 是完全独立, 还是彼此协同, 并不明确, 需要未来更系统的研究予以阐明. WIPI1似乎不参与自噬调控, 但是鉴于其可以有效地结合脂质PI3P, 其可能通过与WIPI2竞争性结合PI3P充当细胞自噬的内源抑制因子. 更重要的是, 在多种肿瘤细胞中, WIPI1的表达水平显著上调或者下调[11,12], 其是否是导致肿瘤细胞自噬紊乱的关键因素也值得探究. WIPI3和WIPI4都可通过结合ATG2A/B调控自噬前体膜的延伸. 然而, 它们的功能是否可以彼此取代, 以及它们是否在细胞自噬中发挥独特的功能仍有待阐明.

随着研究的深入, WIPI2被发现可通过多种不同的作用方式在细胞自噬过程中调控自噬前体膜的延伸, 促进自噬小体的形成. 然而, 除了上述已知的作用模式, WIPI2是否能通过其他未知的机制调控自噬前体膜的延伸, 仍不明确. 此外, WIP2是否调控细胞自噬过程的其他阶段也值得研究. 考虑到WIPI2主要通过结合不同的蛋白发挥不同的调控作用, 利用质谱分析技术系统研究不同刺激条件下WIPI2的相互作用蛋白组, 可能有助于发现WIPI2新的自噬调控功能. 在不同类型的选择性自噬中, WIPI2均需要依赖于自噬前体膜靶向自噬底物, 从而行使功能. 不同的自噬底物, 如入侵病原菌或者受损线粒体, 是否通过相同的分子机制触发WIPI2的招募作用是自噬领域亟待解决的重要问题.

越来越多的自噬相关蛋白被发现参与调控细胞自噬以外的生物学过程, 如细胞周期、DNA损伤反应和核糖体生物发生等[55~60]. WIPI2和其他WIPI蛋白可结合多种不同的脂质分子, 它们的功能很可能并不局限于细胞自噬过程. 然而, 它们在细胞内是否调控自噬以外的生物学过程, 仍然不甚明确. 此外, 与WIPI2突变相关的人类疾病的发生发展是否完全源于WIPI2自噬相关功能的丧失, 也有待阐明. 除了WIPI2, WIPI3和WIPI4突变不仅引起神经系统发育障碍, 还会引发多种神经系统相关疾病, 包括癫痫、自闭症和帕金森病等[61~64]. 值得注意的是, 携带WIPI4突变病人的一个显著特征是铁元素在神经元内的异常累积[61]. 然而, 在其他自噬基因突变相关的疾病中, 异常的铁累积尚未见报道[53], 表明WIPI4突变可能通过干扰自噬以外的生物学过程在相关人类疾病的发生发展中发挥作用.

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Summary for “WIPI2在经典自噬和非经典自噬中的作用”

Emerging roles of WIPI2 in canonical and non-canonical autophagy

Yinfeng Xu[1] & Wei Wan[2*]

[1] *Laboratory of Basic Biology, Hunan First Normal University, Changsha 410205, China;*
[2] *Department of Biochemistry, Zhejiang University School of Medicine, Hangzhou 310058, China*
* Corresponding author, E-mail: wanwei@zju.edu.cn

Macroautophagy (hereafter referred to as autophagy) is a highly conserved lysosome-dependent degradation pathway in eukaryotic cells. Autophagy helps maintain cell homeostasis by eliminating unwanted cellular materials, including protein aggregates, damaged organelles and invading pathogens. Dysfunction of autophagy has been linked to a variety of human diseases, such as cancers, metabolic diseases and neurodegenerative diseases. As a sequential cellular process, autophagy is typically divided into several major steps, including autophagy initiation, membrane nucleation, phagophore expansion, autophagosome formation and autophagosome-lysosome fusion. These distinct autophagy steps are spatiotemporally controlled by a great number of autophagy-related proteins. Among these proteins, WIPI-family proteins, comprised with four members, have been demonstrated to play an important role in phagophore expansion. Mammalian WIPI1 and WIPI2 are highly related to yeast Atg18 while mammalian WIPI3 and WIPI4 are highly close to yeast Atg21. Of note, WIPI2 but not WIPI1 is essential for autophagy, and WIPI3 and WIPI4 are each only partially necessary for autophagy. During canonical autophagy, WIPI2 has been demonstrated to regulate phagophore expansion through several different mechanisms. On the phagophore, PI3P, produced by activated VPS34 complex, and RAB11A mediate the recruitment of WIPI2, which then recruits ATG16L1-ATG5-ATG12 complex by interacting with ATG16L1, resulting in the lipidation of ATG8-family proteins and the expansion of phagophore. Recently, WIPI2 is also shown to promote phagophore expansion by mediating the membrane contact between phagophore and endoplasmic reticulum through interacting with endoplasmic reticulum-localized ULK1 complex. In addition to nutrient starvation-induced canonical autophagy, WIPI2 is required for viral infection-induced non-canonical autophagy, which depends on the activation of cGAS-STING pathway. Interestingly, STING-induced autophagy does not require the upstream autophagic machinery, such as ULK1 complex and VPS34 complex. Stimulated by cytoplasmic DNA, activated cGAS synthesizes cGAMP, which binds to endoplasmic reticulum-localized STING to initiate its intracellular trafficking, leading to STING vesicle formation. By providing membrane sources, STING vesicles act as the primary sites for autophagosome formation. Through a direct interaction, STING brings WIPI2 to STING vesicles to mediate the subsequent recruitment of ATG16L1-ATG5-ATG12 complex, bypassing the upstream autophagic machinery. In response to different cellular or extracellular cues, a variety of WIPI2-based regulation mechanisms are adopted by the cell to tightly control autophagy. Under nutrient-rich conditions, activated mTORC1 phosphorylates WIPI2 to enhance its binding to E3 ubiquitin ligase HUWE1, leading to the proteasomal degradation of WIPI2 and the suppression of autophagy. Upon mitosis induction, activated E3 ubiquitin ligase CRL4 interacts with WIPI2 to promote its ubiquitination and proteasome-dependent degradation, resulting in autophagy inhibition. During bacterial infection, activated TBK1 promotes the recruitment of WIPI2-localized phagophores to bacteria for the initiation of xenophagy and the clearance of bacteria. It is worth noting that a mutation of WIPI2, decreasing its autophagic function, is associated with global developmental abnormalities in humans. In this paper, we aim to review the emerging roles of WIPI2 in the regulation of canonical and non-canonical autophagy.

WIPI2, canonical autophagy, non-canonical autophagy, autophagosome, phagophore

doi: 10.1360/TB-2023-0263