Ubiquitin carboxyl-terminal hydrolase 11 promotes autophagy by de-ubiquitinating and stabilizing Beclin-1

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Abstract

Autophagy is a major degradation process that degrades and recycles cytoplasmic materials through lysosome for maintaining cellular homeostasis. Dysregulated autophagy is linked with numerous human diseases including cancer. Autophagy marker protein B-cell lymphoma-2 interacting protein 1 (Beclin-1) is essential for autophagosome initiation and maturation. Recently, Ubiquitin carboxyl-terminal hydrolase 11 (USP11) has been reported to promote or inhibit autophagy without identification of any direct target. Here through biochemical reaction *in vitro*, we demonstrate that USP11 directly interacts with Beclin-1. Both *in vitro* and *in vivo* de-ubiquitination assays revealed that USP11 de-ubiquitinates Beclin-1. USP11-mediated de-ubiquitination stabilized Beclin-1 and enhanced the formation of the autophagy-specific class III phosphatidylinositol 3-kinase complexes 1 and 2, thereby promoting autophagy. Together, our results demonstrated that USP11 promotes autophagy under unperturbed conditions by de-ubiquitinating and stabilizing Beclin-1 which may serve as a therapeutic target for autophagy-related diseases.

Keywords USP11 · Beclin-1 · Autophagy · De-ubiquitination · Class III phosphatidylinositol 3-kinase complex

Introduction

Autophagy is an evolutionarily conserved cellular recycling process that degrades cytoplasmic proteins, macromolecules, and unwanted organelles through lysosomal degradation to maintain metabolic homeostasis (Rabinowitz & White, 2010; Yang & Klionsky, 2020). In response to different stressors, such as nutrient deprivation and growth factor depletion, autophagy is activated, facilitating cell tolerance

of the microenvironment, which prevents cell damage and promotes survival (White & DiPaola, 2009). In mammalian cells, there are three types of autophagy, namely, chaperone-mediated autophagy (CMA), microautophagy, and macroautophagy (Yang & Klionsky, 2010). In the CMA pathway, proteins containing a KFERQ-like motif are recognized by the chaperone HSC70 and cochaperons and delivered to the lysosome for degradation (Dong et al., 2021; Kaushik & Cuervo, 2018; Yang et al., 2019). In microautophagy, the lysosomal membrane invaginates long-lived proteins into the lysosome for degradation through membrane fission (Schuck, 2020). Macroautophagy has been studied extensively and differs from CMA and microautophagy. In the macroautophagy pathway, autophagic cargos need to be packaged into double-membrane vesicles and subsequently transported to the lysosome (Dikic & Elazar, 2018). In brief, the macroautophagy pathway comprises five stages: phagophore initiation and nucleation, expansion, maturation, fusion with the lysosome, and degradation.

Multiple protein complexes are involved in macroautophagy (Dikic & Elazar, 2018). The autophagy marker protein B-cell lymphoma-2 interacting protein 1 (Beclin-1), the lipid kinase VPS34, and the scaffold protein

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VPS15 constitute core components of the autophagy-specific class III phosphatidylinositol 3-kinase complex (PI3KC3) (Galluzzi & Green, 2019). Beclin-1 recruits one of two positive regulators, ATG14 or UVRAG, in a mutually exclusive manner to serve as the fourth subunit for PI3KC3 complex 1 (PI3KC3-C1) or PI3KC3 complex 2 (PI3KC3-C2), respectively. PI3KC3-C1 is involved in the initiation of autophagosomes and PI3KC3-C2 in the maturation of autophagosomes and endocytosis (Baskaran et al., 2014). During this process, Beclin-1 undergoes multiple functional post-translational modifications, including phosphorylation and ubiquitination. Death-associated protein kinase 1 (DAPK1) phosphorylates Beclin-1 at Thr-119 and reduces Beclin-1 interaction with the apoptosis regulator B-cell lymphoma-2 (Bcl-2), thus promoting autophagy (Zalckvar et al., 2009). The “Lys-11”-linked polyubiquitination of Beclin-1 is mediated by NEDD4 and leads to beclin1 degradation (Platta et al., 2012). Expansion of the CAG (poly-glutamine) repeat in the ATXN3 coding region leads to spinocerebellar ataxia, a clinically and genetically heterogeneous group of cerebellar disorders; whereas normal ATXN3 protein de-ubiquitinates and stabilizes Beclin-1, promoting autophagy, while expansion of the poly-glutamine repeat impairs its function (Ashkenazi et al., 2017).

USP11 has been demonstrated to participate in multiple cellular pathways such as cortical neurogenesis (Chiang et al., 2021), R-loop regulation (Jurga et al., 2021), and DNA repair (Ting et al., 2019). In a proteomic screen, USP11 was identified as a protein that interacts with Beclin-1, VPS34, ATG10, ATG12, and ATG16L1, suggesting that USP11 may function in autophagy (Behrends et al., 2010). Recently, USP11 has been reported to activate autophagy by stabilizing valosin-containing protein (VCP) (Sun et al., 2021). In contrast, Qiao et al. demonstrated that USP11 inhibits autophagy by activating the ERK/mTOR pathway (Qiao et al., 2021). While this manuscript was under preparation, Basic et al. reported that, within the hTERT-immortalized retinal pigment epithelial cell line hTERT RPE-1, USP11 interacts with the PI3KC3-C1 complex and reduces its association with nuclear receptor-binding factor 2 (NRBF2), thus decreasing the lipid kinase activity of PI3KC3-C1 (Basic et al., 2021). Furthermore, USP11 interacts with and stabilizes serine/threonine kinase mTOR, thereby compromising autophagy (Basic et al., 2021). All these studies failed to identify the direct target(s) for USP11, and these conflicting results require clarification.

In this study, we aimed to clarify if USP11 promotes or suppresses autophagy by identification of its direct target(s). We found that USP11 promotes autophagy by directly binding with and de-ubiquitinating Beclin-1, which leads to Beclin-1 stabilization and PI3KC3-1/2 complex formation.

Results

USP11 promotes autophagy in a catalytic activity-dependent manner

Given that the studies before failed to demonstrate if restoring USP11 expression and/or the catalytic activity of USP11 can erase the USP11 depletion-associated autophagic phenotypes, we stably expressed a short hairpin RNA (shRNA) construct targeting the coding region of the USP11 gene in HeLa cells. The resulting USP11 knockdown led to an accumulation of autophagy receptor P62, and there was a decrease in the LC3-II/I ratio on immunoblotting analysis (Fig. 1a) and decreased LC3-II puncta formation on immunocytochemical analysis (Fig. 1b). Re-expression of the shRNA-resistant form of wild type HA-tagged USP11 (HA-USP11), but not the catalytically inactive mutant HA-USP11 (C318S), restored the USP11 depletion-induced changes to P62 levels, the LC3-II/I ratio, and LC3-II puncta formation (Figs. 1a and 1b). Conversely, overexpression of wild type HA-USP11, but not the HA-USP11 (C318S) mutant, increased the LC3-II/I ratio while decreasing P62 protein levels (Fig. 1c). Taken together, our results demonstrated that USP11 promotes autophagy in an enzymatic activity-dependent manner.

USP11 directly interacts with Beclin-1 and the interaction is induced by starvation

Three recent studies failed to identify the direct target of USP11-depletion-mediated autophagy (Basic et al., 2021; Qiao et al., 2021; Sun et al., 2021). We sifted through the interaction networks of the human autophagy system described by Behrends et al. and found candidate interactors of USP11, including Beclin-1 and VPS34 (Behrends et al., 2010). Co-immunoprecipitation assays revealed that endogenous USP11 was present in the immunocomplex of endogenous Beclin-1 in HeLa cells (Fig. 2a), and HA-tagged Beclin-1 (HA-Beclin-1) was present in the FLAG-USP11 immunocomplex in HeLa cells co-expressing HA-Beclin-1 and FLAG-USP11 (Fig. 2b). Using GST pulldown assays, we found that bacterially produced GST-USP11 was able to pull down bacterially produced HIS-Beclin-1 (Fig. 2c), indicating that USP11 directly interacts with Beclin-1. Upon glucose starvation treatment, both exogenous and endogenous interactions between USP11 and Beclin-1 significantly increased (Figs. 2d and 2e). We also found a positive correlation (p -Value < 0.05, R -Value > 0.3) between Beclin-1 and USP11 gene expression in 15 types of tumors from the GEPIA database

Fig. 1 USP11 promotes autophagy in a catalytic activity-dependent manner. **a** USP11 depletion inhibits autophagy. Western blot showing the expression of HA-tagged wild type and enzymatic activity defective mutant (C318S) USP11, P62, and LC3 in normal control (NC) and vector control (VEC) HeLa cells and HeLa cells treated with short hairpin RNA (shRNA) to USP11 (shUSP11). P62 protein levels were normalized to actin. **b** USP11 depletion decreases LC3 puncta formation. LC3 puncta were measured by immunofluorescence assay

and further quantified by ImageJ software. A two-tailed Student's *t* test was used to determine the statistical significance of group differences. **p* < 0.05, ***p* < 0.01, *****p* < 0.001. **c** USP11 overexpression promotes autophagy. Western blot showing the expression of HA-tagged wild type and enzymatic activity defective mutant (C318S) USP11, P62, and LC3 in normal HeLa cells. P62 protein levels were normalized to actin

(<http://gepia.cancer-pku.cn>) (Fig. 2f). Therefore, we concluded that USP11 directly interacts with Beclin-1, and the interaction is induced by starvation.

USP11 de-ubiquitinates and stabilizes Beclin-1

Given that USP11 directly interacts with Beclin-1 (Fig. 2), we reasoned that Beclin-1 could be a substrate for USP11. To test this hypothesis, we co-expressed FLAG-Beclin-1 and HA-tagged ubiquitin (HA-Ub) along with Myc-USP11 or catalytically inactive mutant Myc-USP11 (C318S) in HEK293T cells. Co-expressing Myc-USP11 greatly reduced the ubiquitination levels of Beclin-1, whereas co-expressing Myc-USP11 (C318S) did not have any obvious impact on the ubiquitination levels of Beclin-1 (Fig. 3a). This enzymatic activity-dependent de-ubiquitination mediated by USP11 was also observed on endogenous Beclin-1 (Fig. 3b). Furthermore, inhibition of USP11 expression by siRNA increased Beclin-1 ubiquitination (Fig. 3c). In vitro

de-ubiquitination assays showed that bacterially produced GST-USP11, but not GST-USP11 (C318S), efficiently removed the polyubiquitin chains from FLAG-Beclin-1, which was enriched by immunoprecipitation with an anti-FLAG antibody from HEK293T cells co-expressing FLAG-Beclin-1 and HA-Ub (Fig. 3d). Thus, we concluded that USP11 de-ubiquitinates Beclin-1.

Reportedly, Beclin-1 ubiquitination is a target for proteasomal degradation (Han et al., 2018; Li et al., 2021; Pei et al., 2017). We thus speculated that the USP11-mediated de-ubiquitination of Beclin-1 stabilizes the protein. Indeed, when we inhibited USP11 expression in HeLa cells by shRNA treatment, Beclin-1 protein levels reduced, and this reduction was rescued by the re-expression of wild type HA-USP11, but not the catalytically inactive mutant HA-USP11 (C318S) (Fig. 3e). This USP11-depletion-induced reduction of Beclin-1 protein levels was also restored by pretreating the cells with MG-132—a potent cell-permeable proteasome inhibitor (Fig. 3f). We also found that the USP11-knockdown-induced

Fig. 2 USP11 directly interacts with Beclin-1 and this interaction is induced by starvation. **a** Endogenous USP11 interacts with Beclin-1. Western blot showing the endogenous USP11 in the anti-Beclin-1 immunoprecipitates but not the anti-IgG (negative control) immunoprecipitates from HEK293T cells. **b** Ectopic tagged USP11 interacts with Beclin-1. Western blot showing the HA-Beclin-1 in the anti-FLAG immunoprecipitates from HEK293T cells overexpressing HA-Beclin-1 and with or without FLAG-USP11. **c** USP11 directly interacts with Beclin-1. Western blot showing the bacterial produced His-Beclin-1 in the anti-GST immunoprecipitates that containing

GST-USP11 but not the GST (negative control) group. **d,e.** Starvation stimulates USP11 interaction with Beclin-1. Before harvest, cell culture medium was removed and the cells were washed with PBS for 3 times and then emersed in fresh medium without glucose. Total cell lysates were extracted and subjected to immunoprecipitation with anti-FLAG (**d**) or anti-Beclin-1 (**e**) antibody followed by immunoblotting with antibodies as indicated. **f** Data were collected from GEPIA database (<http://gepia.cancer-pku.cn/>); 15 types of tumor (33 types of tumor in total) exhibited gene correlation (R -value > 3 , p value < 0.05) between Beclin-1 and USP11

reduction in Beclin-1 protein levels occurred both under unperturbed and glucose-starvation-induced autophagy conditions (Fig. 3g). Our findings therefore indicate that Beclin-1 is a substrate of USP11, and USP11-mediated de-ubiquitination stabilizes Beclin-1.

USP11 promotes the formation of autophagy-specific PI3KC3 complexes

The scaffolding protein Beclin-1 brings in one of two positive regulators, ATG14 or UVRAG, to serve as the

It was reported that, without rescue experiments, the inhibition of USP10 or USP13 expression by three independent siRNA oligos destabilized Beclin-1, whereas overexpressing USP10 or USP13, but not the catalytically inactive mutant, reduced Beclin-1 ubiquitination *in vivo* (Liu et al., 2011). While this manuscript was under preparation, another group also found evidence that USP11 de-ubiquitinates and stabilizes Beclin-1 to promote autophagy and subsequent autophagy-induced ferroptosis (Rong et al., 2021). These studies, along with previously mentioned reports of autophagy modulation by USP11, failed to provide *in vitro* evidence of a direct interaction between USP11 and Beclin-1.

Fig. 4 USP11 promotes formation of autophagy-specific PI3KC3 complexes. **a** USP11-depletion decreases Beclin-1 interaction with other subunits of autophagy-specific class III phosphatidylinositol 3-kinase complexes (PI3KC3). Western blot showing the VPS15, VPS34, ATG14 and UVRAG in the anti-FLAG immunoprecipitates from shUSP11 HeLa cell that reconstituted with HA-tagged vector control, wild type, enzymatic activity defective mutant (C318S)

USP11 and with or without FLAG-Beclin-1. **b** USP11-overexpression promotes Beclin-1 interaction with other subunits of PI3KC3. Western blot showing the VPS15, VPS34, ATG14 and UVRAG in the anti-FLAG immunoprecipitates from HEK293T cells that over-expressing HA-tagged vector control, wild type, enzymatic activity defective mutant (C318S) USP11 and with or without FLAG-Beclin-1

or positive in vitro de-ubiquitination assays of USP11/Beclin-1 (Basic et al., 2021; Qiao et al., 2021; Sun et al., 2021). Thus, these studies could not exclude the possibility that USP11-mediated autophagy is executed through its interacting de-ubiquitinases such as USP4 (L. Zhang et al., 2012), USP7 (Georges et al., 2018), USP15 (Sowa et al., 2009), and OTUD5 (Sowa et al., 2009). In this study, we have provided strong evidence to support the hypothesis that USP11 promotes autophagy by directly de-ubiquitinating and stabilizing Beclin-1. These lines of evidence include results that show (1) USP11 directly interacts with Beclin-1; (2) USP11 de-ubiquitinates Beclin-1 in vitro; and (3) the USP11-mediated de-ubiquitination/stabilization of Beclin-1 and formation of the pro-autophagy PI3KC3 complexes are rescued by the re-expression of wild type USP11, but not the catalytically inactive mutant.

Upregulated USP11 expression has been reported in patient cohorts of gastric cancer (Liu et al., 2021), breast cancer (Bayraktar et al., 2013; Zhou et al., 2017), estrogen receptor alpha-positive breast cancer (Dwane et al., 2020), ovarian cancer (Wang et al., 2019), hepatocellular carcinoma (Zhang et al., 2018), and colorectal cancer (Huang et al., 2021). In addition, high USP11 expression correlated with shorter survival times in patient cohorts of breast

cancer (Bayraktar et al., 2013; Garcia et al., 2018; Zhou et al., 2017), estrogen receptor alpha-positive breast cancer (Dwane et al., 2020), ovarian cancer (Wang et al., 2019), hepatocellular carcinoma (Zhang et al., 2018), and colorectal cancer (Huang et al., 2021). We also investigated the relationship between USP11 and Beclin-1 gene expression in 33 types of TCGA tumors from GEPIA database, and there was a positive correlation between the genes in 15 of the tumor types, each exhibiting R values greater than 3. These results suggest that USP11 may functionally correlate with Beclin-1 in tumor cells.

Several studies have demonstrated that autophagy acts to promote tumor survival and growth in advanced cancers (Dikic & Elazar, 2018), and after exploring the GEPIA database (<http://gepia.cancer-pku.cn/>), we discovered that high Beclin-1 expression correlates with shorter survival times in patient cohorts of ovarian cancer, kidney chromophobe, and lower grade brain glioma (Fig. S1). This suggests that autophagy may be linked to the poor prognosis following some tumor therapeutics. Moreover, the USP11-mediated stabilization of VCP activated autophagy and conferred colorectal cancer resistance to fluorouracil treatment (Sun et al., 2021). Taken together, we demonstrate that Beclin-1 is a direct target for USP11 and USP11-mediated

de-ubiquitination and stabilization of Beclin-1 leads to assembling of PI3KC3-C1 and PI3KC3-C2 complex, ultimately promoting autophagy. Therefore, targeting USP11 for inhibition could serve as a therapeutic strategy for autophagy-related diseases.

Materials and methods

Cell culture

Human HEK293T and HeLa cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin in an atmosphere containing 5% CO₂ at 37 °C.

Antibodies

The following antibodies were used in this study: Anti-FLAG M2(F3165, Sigma), Anti-Beclin-1 (A302-566A/567A, Bethyl), Anti-HA Tag (AE036, ABclonal), Anti-VPS15 (A302-571A, Bethyl), Anti-VPS34 (A12295, ABclonal), Anti-UVRAG (A8462, ABclonal), Anti-LC3 (A19665, ABclonal), Anti-P62 (A302-856A, Bethyl), Anti-Actin (AC026, ABclonal), Anti-His (D2913, MBL), Anti-GST (M2093, MBL), Anti-USP11 (A301-613A), Anti-ATG14 (A7526, ABclonal).

Glucose starvation

For starvation induction, glucose-free medium (11,966–025, Gibco) were used. Before harvested, cultured cells were quickly washed with PBS for 3 times and added with glucose-free medium for indicated time.

Plasmids and transfection

A full-length cDNA clone encoding human Beclin-1 was amplified by PCR and subcloned into the pcDNA3.0 3XFLAG vector and pcDNA3.0 3xHA vector. Wild type USP11 cDNA was cloned into the pcDNA3.0-HA vector, pcDNA3.0-FLAG vector and pcDNA3.1-Myc vector. The cDNA of Ubiquitin was cloned into the pcDNA3.1-Myc vector. Point mutations in the catalytically inactive USP11 (C318S) gene were generated using the Mut Express II Fast Mutagenesis Kit V2 (Vazyme, Nanjing, China). The siRNA oligonucleotide against USP11 (si-USP11 sequence: CUG AAAUGAAAAAAGGUA) were purchased from Guangzhou RiboBio. The shRNA sequence targeting USP11 was 5'-TCGCGGTTTCCAACCATTATG-3'. Bacteria expressing His-tagged Beclin-1 and GST-tagged USP11 were generated using the pET28a (Invitrogen) and pGEX-4T-1 (GE

Healthcare) systems. Plasmid transfections were performed with polyethyleneimine (PEI).

Undenatured co-immunoprecipitation

Cells were harvested and lysed with lysis buffer (150 mM NaCl; 50 mM Tris, pH 7.5; 0.5% NP-40; 5 mM EDTA) at 4 °C for 30 min. Further the lysates were centrifuge with 13000g to collect the supernatant for immunoprecipitation with antibody at 4 °C overnight. Then, protein A/G magnetic beads were added to the cell lysates and incubated at 4 °C for 1 h. The beads were washed with cold lysis buffer three times, for 5 min each time, on a rotating mixer.

Denatured co-immunoprecipitation

Immunoprecipitation assays in denaturing conditions (SDS-IP) were executed as previously described (Yoo et al., 2014). Briefly, the harvested cells were lysed with lysis buffer (150 mM Tris, pH 8.0, 5% SDS, 30% glycerol) at 100°C for 10 min before being digested with benzonuclease (Sigma) at room temperature for 30 min. After centrifugation (13000g, 10 min), the supernatant was diluted with Buffer A (50 mM Tris-HCl [pH 8], 150 mM NaCl, 1% Triton X-100, 1×protease inhibitor cocktail, and 2 mM NEM) to 20 times dilution and immunoprecipitated with appropriate antibodies.

De-ubiquitination in vitro

Cells transfected with FLAG-Beclin-1 and HA-Ub were harvested 48 h after transfection and lysed with 400 mM NaCl washing buffer (400 mM NaCl; 50 mM Tris, pH 7.5; 0.5% NP-40; 5 mM EDTA). Flag-Beclin-1 protein and its modified forms were pulled down with FLAG-M2 beads. After washing with 400 mM washing buffer three times, the beads were incubated with GST/GST-USP11/GST-USP11 (C318S) in de-ubiquitination buffer (50 mM Tris-HCl [pH 7.4], 50 mM MgCl₂, 1 mM DTT) at 37 °C for 1 h. After incubation, the beads were washed with 400 mM washing buffer three times.

Immunofluorescence

HeLa cells were washed with PBS three times and fixed with 4% paraformaldehyde at room temperature for 10 min. Then the cells were permeabilized with 0.5% Triton X-100 in PBS for 5 min. After permeabilization, the cells were blocked with 2% BSA in PBST at room temperature for 30 min and further incubated with the primary antibody overnight followed by the secondary antibody for 1 h. After washing with PBST three times, DAPI was added, and the coverslips (CIT-OGLAS) were covered with antifade. And images were captured with OLYMPUS fluorescence microscope (CKX53).

Western blot

Total protein from cells was extracted with 150 mM NaCl lysis buffer as described earlier. Samples were separated using SDS-PAGE and transferred to PVDF membranes. After skim milk blocking, the membranes were incubated with primary antibody at 4 °C overnight. The membranes were incubated with secondary antibodies for 1 h at room temperature, then visualized using electro chemiluminescence detection reagents (SuperSignal™ West Pico PLUS, Thermo, lot#34,580).

Gene correlation

Gene correlation between USP11 and Beclin-1 in tumors was analyzed online by GEPIA (<http://gepia.cancer-pku.cn>). This website performs pair-wise gene expression correlation analysis for given sets of TCGA expression data, using Pearson method. Cases that p -Value < 0.05, R -Value > 0.3 were selected as convincing correlation between USP11 and Beclin-1.

Kaplan–Meier survival analysis

The survival analysis is done online (<http://gepia.cancer-pku.cn>) to analyze correlations between levels of Beclin-1 with overall survival (OS) in cancers. All survival diagrams shown in the manuscript were analyzed by GEPIA using Log-rank test, a.k.a the Mantel–Cox test.

Statistical analysis

Data were analyzed by Prism 7.0 software (GraphPad Prism). A two-tailed Student's t test was used to determine the statistical significance. All data are presented as the means $\pm$ s.d. or means $\pm$ s.e.m. as indicated, and a p value of < 0.05 was considered statistically significant.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s42764-022-00061-6>.

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Data availability All data generated or analyzed during this study are included in this manuscript. Materials described in this manuscript are available upon request.

Code availability Not applicable.

Declarations

Conflicts of interest The authors declare no potential conflicts of interest.

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References

- Ashkenazi, A., Bento, C. F., Ricketts, T., Vicinanza, M., Siddiqi, F., Pavel, M., Squitieri, F., Hardenberg, M. C., Imarisio, S., Menzies, F. M., & Rubinsztein, D. C. (2017). Polyglutamine tracts regulate beclin 1-dependent autophagy. *Nature*, 545(7652), 108–111. <https://doi.org/10.1038/nature22078>
- Basic, M., Hertel, A., Bajdzienko, J., Bonn, F., Tellechea, M., Stolz, A., Kern, A., Behl, C., & Bremm, A. (2021). The deubiquitinase USP11 is a versatile and conserved regulator of autophagy. *Journal of Biological Chemistry*, 297(5), 101263. <https://doi.org/10.1016/j.jbc.2021.101263>
- Baskaran, S., Carlson, L. A., Stjepanovic, G., Young, L. N., Kim, D. J., Grob, P., Stanley, R. E., Nogales, E., & Hurley, J. H. (2014). Architecture and dynamics of the autophagic phosphatidylinositol 3-kinase complex. *Elife*, 3. doi:<https://doi.org/10.7554/eLife.05115>
- Bayraktar, S., Gutierrez Barrera, A. M., Liu, D., Pusztai, L., Litton, J., Valero, V., Hunt, K., Hortobagyi, G. N., Wu, Y., Symmans, F., & Arun, B. (2013). USP-11 as a predictive and prognostic factor following neoadjuvant therapy in women with breast cancer. *Cancer Journal*, 19(1), 10–17. <https://doi.org/10.1097/PPO.0b013e3182801b3a>
- Behrends, C., Sowa, M. E., Gygi, S. P., & Harper, J. W. (2010). Network organization of the human autophagy system. *Nature*, 466(7302), 68–76. <https://doi.org/10.1038/nature09204>
- Chiang, S. Y., Wu, H. C., Lin, S. Y., Chen, H. Y., Wang, C. F., Yeh, N. H., Shih, J. H., Huang, Y. S., Kuo, H. C., Chou, S. J., & Chen, R. H. (2021). Usp11 controls cortical neurogenesis and neuronal migration through Sox11 stabilization. *Sci Adv*, 7(7). <https://doi.org/10.1126/sciadv.abc6093>
- Dikic, I., & Elazar, Z. (2018). Mechanism and medical implications of mammalian autophagy. *Nature Reviews Molecular Cell Biology*, 19(6), 349–364. <https://doi.org/10.1038/s41580-018-0003-4>
- Dong, S., Wang, Q., Kao, Y. R., Diaz, A., Tasset, I., Kaushik, S., Thiruthuvanathan, V., Zintiridou, A., Nieves, E., Dzieciatkowska, M., Reisz, J. A., Gavathiotis, E., D'Alessandro, A., Will, B., & Cuervo, A. M. (2021). Chaperone-mediated autophagy sustains haematopoietic stem-cell function. *Nature*, 591(7848), 117–123. <https://doi.org/10.1038/s41586-020-03129-z>
- Dwane, L., O'Connor, A. E., Das, S., Moran, B., Mulrane, L., Pinto-Fernandez, A., Ward, E., Blumel, A. M., Cavanagh, B.

- L., Mooney, B., Dirac, A. M., Jirstrom, K., Kessler, B. M., Ni Chonghaile, T., Bernards, R., Gallagher, W. M., & O'Connor, D. P. (2020). A functional genomic screen identifies the deubiquitinase USP11 as a novel transcriptional regulator of ERalpha in breast cancer. *Cancer Research*, 80(22), 5076–5088. <https://doi.org/10.1158/0008-5472.CAN-20-0214>
- Galluzzi, L., & Green, D. R. (2019). Autophagy-independent functions of the autophagy machinery. *Cell*, 177(7), 1682–1699. <https://doi.org/10.1016/j.cell.2019.05.026>
- Garcia, D. A., Baek, C., Estrada, M. V., Tysl, T., Bennett, E. J., Yang, J., & Chang, J. T. (2018). USP11 Enhances TGFbeta-induced epithelial-mesenchymal plasticity and human breast cancer metastasis. *Molecular Cancer Research*, 16(7), 1172–1184. <https://doi.org/10.1158/1541-7786.MCR-17-0723>
- Georges, A., Marcon, E., Greenblatt, J., & Frappier, L. (2018). Identification and characterization of USP7 targets in cancer cells. *Science and Reports*, 8(1), 15833. <https://doi.org/10.1038/s41598-018-34197-x>
- Han, T., Guo, M., Gan, M., Yu, B., Tian, X., & Wang, J. B. (2018). TRIM59 regulates autophagy through modulating both the transcription and the ubiquitination of BECN1. *Autophagy*, 14(12), 2035–2048. <https://doi.org/10.1080/15548627.2018.1491493>
- Huang, Y. Y., Zhang, C. M., Dai, Y. B., Lin, J. G., Lin, N., Huang, Z. X., & Xu, T. W. (2021). USP11 facilitates colorectal cancer proliferation and metastasis by regulating IGF2BP3 stability. *Am J Transl Res*, 13(2), 480–496.
- Jurga, M., Abugable, A. A., Goldman, A. S. H., & El-Khamisy, S. F. (2021). USP11 controls R-loops by regulating senataxin proteostasis. *Nature Communications*, 12(1), 5156. <https://doi.org/10.1038/s41467-021-25459-w>
- Kaushik, S., & Cuervo, A. M. (2018). The coming of age of chaperone-mediated autophagy. *Nature Reviews Molecular Cell Biology*, 19(6), 365–381. <https://doi.org/10.1038/s41580-018-0001-6>
- Li, X., Yang, K. B., Chen, W., Mai, J., Wu, X. Q., Sun, T., Wu, R. Y., Jiao, L., Li, D. D., Ji, J., Zhang, H. L., Yu, Y., Chen, Y. H., Feng, G. K., Deng, R., Li, J. D., & Zhu, X. F. (2021). CUL3 (cullin 3)-mediated ubiquitination and degradation of BECN1 (beclin 1) inhibit autophagy and promote tumor progression. *Autophagy*, 1–18. <https://doi.org/10.1080/15548627.2021.1912270>
- Liu, H., Liu, M., He, B., & Li, Q. (2021). Inhibition of USP11 sensitizes gastric cancer to chemotherapy via suppressing RhoA and Ras-mediated signaling pathways. *Clin Res Hepatol Gastroenterol*, 101779. <https://doi.org/10.1016/j.clinre.2021.101779>
- Liu, J., Xia, H., Kim, M., Xu, L., Li, Y., Zhang, L., Cai, Y., Norberg, H. V., Zhang, T., Furuya, T., Jin, M., Zhu, Z., Wang, H., Yu, J., Li, Y., Hao, Y., Choi, A., Ke, H., Ma, D., & Yuan, J. (2011). Beclin1 controls the levels of p53 by regulating the deubiquitination activity of USP10 and USP13. *Cell*, 147(1), 223–234. <https://doi.org/10.1016/j.cell.2011.08.037>
- Pei, G., Buijze, H., Liu, H., Moura-Alves, P., Goosmann, C., Brinkmann, V., Kawabe, H., Dorhoi, A., & Kaufmann, S. H. E. (2017). The E3 ubiquitin ligase NEDD4 enhances killing of membrane-perturbing intracellular bacteria by promoting autophagy. *Autophagy*, 13(12), 2041–2055. <https://doi.org/10.1080/15548627.2017.1376160>
- Platta, H. W., Abrahamsen, H., Thoresen, S. B., & Stenmark, H. (2012). Nedd4-dependent lysine-11-linked polyubiquitination of the tumour suppressor Beclin 1. *The Biochemical Journal*, 441(1), 399–406. <https://doi.org/10.1042/BJ20111424>
- Qiao, L., Zhang, Q., Sun, Z., Liu, Q., Wu, Z., Hu, W., Bao, S., Yang, Q., & Liu, L. (2021). The E2F1/USP11 positive feedback loop promotes hepatocellular carcinoma metastasis and inhibits autophagy by activating ERK/mTOR pathway. *Cancer Letters*, 514, 63–78. <https://doi.org/10.1016/j.canlet.2021.05.015>
- Rabinowitz, J. D., & White, E. (2010). Autophagy and metabolism. *Science*, 330(6009), 1344–1348. <https://doi.org/10.1126/science.1193497>
- Rong, Y., Fan, J., Ji, C., Wang, Z., Ge, X., Wang, J., Ye, W., Yin, G., Cai, W., & Liu, W. (2021). USP11 regulates autophagy-dependent ferroptosis after spinal cord ischemia-reperfusion injury by deubiquitinating Beclin 1. *Cell Death and Differentiation*. <https://doi.org/10.1038/s41418-021-00907-8>
- Schuck, S. (2020). Microautophagy - distinct molecular mechanisms handle cargoes of many sizes. *J Cell Sci*, 133(17). <https://doi.org/10.1242/jcs.246322>
- Sowa, M. E., Bennett, E. J., Gygi, S. P., & Harper, J. W. (2009). Defining the human deubiquitinating enzyme interaction landscape. *Cell*, 138(2), 389–403. <https://doi.org/10.1016/j.cell.2009.04.042>
- Sun, H., Wang, R., Liu, Y., Mei, H., Liu, X., & Peng, Z. (2021). USP11 induce resistance to 5-fluorouracil in colorectal cancer through activating autophagy by stabilizing VCP. *Journal of Cancer*, 12(8), 2317–2325. <https://doi.org/10.7150/jca.52158>
- Ting, X., Xia, L., Yang, J., He, L., Si, W., Shang, Y., & Sun, L. (2019). USP11 acts as a histone deubiquitinase functioning in chromatin reorganization during DNA repair. *Nucleic Acids Research*, 47(18), 9721–9740. <https://doi.org/10.1093/nar/gkz726>
- Wang, W., Wang, J., Yan, H., Zhang, K., & Liu, Y. (2019). Upregulation of USP11 promotes epithelial-to-mesenchymal transition by deubiquitinating Snail in ovarian cancer. *Oncology Reports*, 41(3), 1739–1748. <https://doi.org/10.3892/or.2018.6924>
- White, E., & DiPaola, R. S. (2009). The double-edged sword of autophagy modulation in cancer. *Clinical Cancer Research*, 15(17), 5308–5316. <https://doi.org/10.1158/1078-0432.CCR-07-5023>
- Yang, Q., Wang, R., & Zhu, L. (2019). Chaperone-mediated autophagy. *Advances in Experimental Medicine and Biology*, 1206, 435–452. https://doi.org/10.1007/978-981-15-0602-4_20
- Yang, Y., & Klionsky, D. J. (2020). Autophagy and disease: unanswered questions. *Cell Death and Differentiation*, 27(3), 858–871. <https://doi.org/10.1038/s41418-019-0480-9>
- Yang, Z., & Klionsky, D. J. (2010). Mammalian autophagy: core molecular machinery and signaling regulation. *Current Opinion in Cell Biology*, 22(2), 124–131. <https://doi.org/10.1016/j.ccb.2009.11.014>
- Zalcckvar, E., Berissi, H., Mizrachy, L., Idelchuk, Y., Koren, I., Eisenstein, M., Sabanay, H., Pinkas-Kramarski, R., & Kimchi, A. (2009). DAP-kinase-mediated phosphorylation on the BH3 domain of beclin 1 promotes dissociation of beclin 1 from Bcl-XL and induction of autophagy. *EMBO Reports*, 10(3), 285–292. <https://doi.org/10.1038/embor.2008.246>
- Zhang, L., Zhou, F., Drabsch, Y., Gao, R., Snaar-Jagalska, B. E., Mickanin, C., Huang, H., Sheppard, K. A., Porter, J. A., Lu, C. X., & ten Dijke, P. (2012). USP4 is regulated by AKT phosphorylation and directly deubiquitylates TGF-beta type I receptor. *Nature Cell Biology*, 14(7), 717–726. <https://doi.org/10.1038/ncb2522>
- Zhang, S., Xie, C., Li, H., Zhang, K., Li, J., Wang, X., & Yin, Z. (2018). Ubiquitin-specific protease 11 serves as a marker of poor prognosis and promotes metastasis in hepatocellular carcinoma. *Laboratory Investigation*, 98(7), 883–894. <https://doi.org/10.1038/s41374-018-0050-7>
- Zhou, Z., Luo, A., Shrivastava, I., He, M., Huang, Y., Bahar, I., Liu, Z., & Wan, Y. (2017). Regulation of XIAP turnover reveals a role for USP11 in promotion of tumorigenesis. *eBioMedicine*, 15, 48–61. <https://doi.org/10.1016/j.ebiom.2016.12.014>