The inflammation repressor TNIP1/ABIN-1 is degraded by autophagy following TBK1 phosphorylation of its LIR

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Abstract

The inflammatory repressor TNIP1/ABIN-1 is important for keeping in check inflammatory and cell-death pathways to avoid potentially dangerous sustained activation of these pathways. We have now found that TNIP1 is rapidly degraded by selective macroautophagy/autophagy early (0-4 h) after activation of TLR3 by poly(I:C)-treatment to allow expression of pro-inflammatory genes and proteins. A few hours later (6 h), TNIP1 levels rise again to counteract sustained inflammatory signalling. TBK1-mediated phosphorylation of a TNIP1 LIR motif regulates selective autophagy of TNIP1 by stimulating interaction with Atg8-family proteins. This is a novel level of regulation of TNIP1, whose protein level is crucial for controlling inflammatory signalling.

KEYWORDS ABIN-1; Atg8; autophagy; inflammation; LIR; TBK1; TNIP1

Autophagy is triggered by a plethora of stressors and is important for maintaining cell homeostasis. Unsurprisingly, autophagy plays a regulatory role in the innate immune response. Until now, autophagy has mainly been shown to counteract infection and to limit excessive inflammation, by targeting pathogenic factors and immune signaling mediators for degradation. However, as with cancer, it appears that the role of autophagy in the innate immune response can be context dependent. In a recent study, we show that the anti-inflammatory adaptor protein TNIP1 is degraded during the early stages of inflammatory signaling, which allows the establishment of a robust inflammatory response [1]. TNIP1 has been implicated in preventing excessive inflammation by targeting the NFKB/NF-κB pathway and regulating inflammation-induced cell death. *Tnip1* knockout mice die during embryogenesis due to TNF-induced liver inflammation. In human genome-wide association studies, TNIP1 is one of the highest scoring non-MHC genes associated with a wide range of autoimmune diseases.

In a quantitative mass spectrometry-based proteomic screen aimed at identifying potential autophagy regulators, we found that a ubiquitinated form of TNIP1 accumulates upon blocking lysosomal degradation. Further experiments confirm that TNIP1 is degraded in the lysosome under basal conditions, with the proteasome playing a negligible role. However, only a fraction of TNIP1 is ubiquitinated and ubiquitination is not required for lysosomal degradation of TNIP1. Because TNIP1 plays an important role in regulating inflammatory signaling, we pursued unraveling the mechanisms involved in TNIP1 degradation. In the TNIP1 interactome, we found several known autophagy receptors, including SQSTM1, NBR1, TAX1BP1 and OPTN. Upon blockage of lysosomal acidification, we observed by confocal microscopy that TNIP1 accumulates along with SQSTM1, TAX1BP1 and CALCOCO2/NDP52. Interestingly, TNIP1 no longer shows BafA1-induced accumulation in penta knockout (KO) cells (KO of *SQSTM1/p62*, *NBR1*, *TAX1BP1*, *CALCOCO2/NDP52* and *OPTN*). Single KO of *SQSTM1* and *OPTN* each lead to a reduction in TNIP1 accumulation upon blockage of lysosomal acidification, suggesting that under basal conditions TNIP1 lysosomal turnover is aided by SQSTM1-like receptors (SLRs). Furthermore, measurement of TNIP1 autophagic flux using tandem-fluorescently tagged TNIP1 shows that *ATG7* and *ATG16L1* KO only partially block TNIP1 turnover, while this is strongly reduced or blocked in *RB1CC1/FIP200* and *ATG9* KO cells. Together, this shows that TNIP1 is degraded in lysosomes through both ATG7-dependent and -independent pathways.

Considering that TNIP1 is mainly a lysosomal substrate, we wondered whether TNIP1 may also have the characteristics of an autophagy receptor, which includes a region for binding

Atg8-family proteins. The human family of Atg8 proteins consists of the LC3 subfamily with MAP1LC3A/LC3A, LC3B, LC3B2, and LC3C, and of the GABARAP subfamily with GABARAP, GABARAPL1 and GABARAPL2. An unbiased peptide array screen of TNIP1, reveals two potential LC3-interacting regions (LIRs) that both correspond to the core LIR sequence of (W/F/Y)xx(V/I/L). In vitro GST-affinity-isolation assays show that TNIP1 binds to all human Atg8-family proteins, but poorly to GABARAPL2 and with the highest affinity for GABARAP. Mutating the two LIRs, LIR1 (amino acids 83-86) and LIR2 (amino acids 125-128), reveals that LIR2 is mainly responsible for the binding, but by mutating both LIRs, almost all binding is lost.

Sustained inflammatory signaling has been reported in cell lines and mice with a KO for *Tnip1*. KO of *TNIP1* in our HeLa cell system leads to upregulation of several pro-inflammatory genes. This effect is rescued equally well by re-expression of either wild-type or LIR-mutant TNIP1. The observed reversal of inflammatory activation is dependent on the presence of TNIP1, but not its LIR-dependent turnover. To further elucidate the functional significance of the LIRs and the lysosomal degradation of TNIP1, we examined the effect of inflammatory stimuli on TNIP1 degradation. To this end, we used poly(I:C), a double-strand RNA mimic that triggers an inflammatory response via TLR3 signaling, when taken up by endocytosis. We observe a gradual degradation of TNIP1 during the first 4 h of stimuli, after which the levels of TNIP1 increase again at 6 h due to increased transcription. Concomitant with the decrease in TNIP1 protein, pro-inflammatory proteins (e.g., ISG15 and the chemokine CCL5) increase. The initial degradation of TNIP1 is dependent on canonical autophagy as it is blocked by KO of *ATG7*, *ATG101* or *RB1CC1/FIP200*, but independent of the SLRs, which is in contrast to the basal turnover of TNIP1. Proteasomal inhibition does not stabilize TNIP1 levels. The pro-inflammatory signaling induced by poly(I:C) leads to a LIR-dependent autophagic degradation of TNIP1 as shown by reconstitution of *TNIP1* KO cells with wild-type and LIR mutant TNIP1. We also observe increased co-immunoprecipitation of wild-type TNIP1 with LC3B following poly(I:C) stimulation of cells relative to LIR mutant TNIP1. From our TNIP1 interactome, we identified TBK1 (TANK binding kinase 1), which is part of the signaling cascade triggered by poly(I:C), as a possible interactor of TNIP1. We further observed that activated TBK1 colocalizes with TNIP1 in poly(I:C) stimulated cells, making us speculate that TBK1 may be involved in the induced degradation of TNIP1. Using MS-based phosphoproteomics we identified phosphorylated serine residues N-terminal to LIR2. Phosphomimetic mutants of these serines of TNIP1 bind Atg8-family proteins with up to 6-fold

higher affinity. Inhibition of TBK1 activity with the inhibitor MRT67307 reduces interaction of TNIP1 with LC3B. The poly(I:C)-induced turnover of TNIP1 is abrogated by inhibition of TBK1 and does not occur in *TBK1* KO cells.

In conclusion, our study demonstrates that the negative regulator of inflammatory signalling, TNIP1, is degraded by autophagy shortly after activation of TLR3 by poly(I:C), to allow a robust inflammatory response (Figure 1). Subsequently, increased transcription of *TNIP1* ensures that the protein comes back and can inhibit a sustained and potentially damaging inflammatory response. The autophagic degradation of TNIP1 is induced by TBK1-mediated phosphorylation of the LIR increasing binding of Atg8-family proteins. In analogy to OPTN, recruitment of core autophagy proteins can also presumably contribute to efficient autophagy of TBK1-phosphorylated TNIP1.

Disclosure statement

The authors declare that they have no conflict of interest.

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Reference

1. Zhou J, Rasmussen NL, Olsvik HL, Akimov V, Hu Z, Evjen G, Kaeser-Pebernard S, Sankar DS, Roubaty C, Verlhac P, et al. TBK1 phosphorylation activates LIR-dependent degradation of the inflammation repressor TNIP1. J Cell Biol. 2023 Feb 6;222(2).

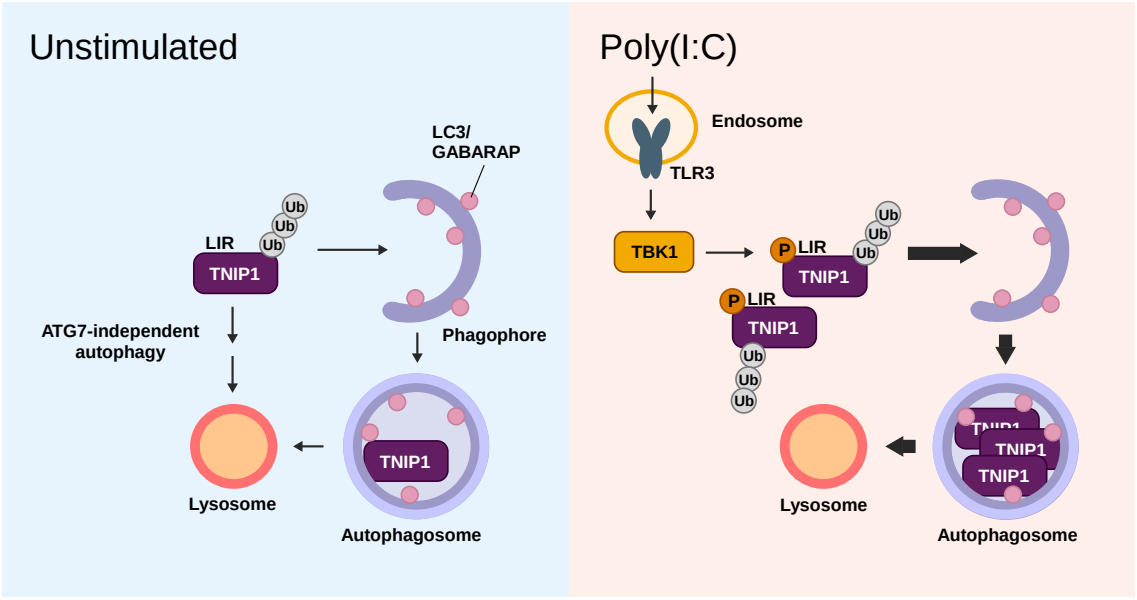
Unstimulated
LC3/
GABARAP
LIR
Ub
Ub
Ub
TNIP1
ATG7-independent
autophagy
Phagophore
TNIP1
Lysosome
Autophagosome
Poly(I:C)
Endosome
TLR3
TBK1
P
LIR
Ub
Ub
Ub
TNIP1
P
LIR
TNIP1
Ub
Ub
Ub
Lysosome
TNIP1
TNIP1
TNIP1
Autophagosome

FIGURE LEGEND

Figure 1. Different modes of autophagic degradation of TNIP1 in unstimulated and TLR3-stimulated conditions. In unstimulated cells, TNIP1 is degraded by both ATG7-dependent and -independent (non-canonical) pathways (left panel). Shortly (0-4 h) after stimulation of TLR3 by poly(I:C), TBK1 phosphorylates LIR2 of TNIP1 to induce efficient degradation of TNIP1 by Atg8-family- and ATG7-dependent canonical macroautophagy to elicit a potent inflammatory response (right panel).