

OTULIN deficiency causes auto-inflammatory syndrome

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Abstract

Ubiquitin chains assembled via the N-terminal methionine (Met1; or linear ubiquitin), conjugated by the linear ubiquitin chain assembly complex (LUBAC), participate in NF- κ B dependent inflammatory signaling and immune responses. A recent report in *Cell* finds that OTULIN, a deubiquitinase that selectively cleaves Met1-linked ubiquitin chains, is essential for restraining inflammation in vivo.

Ubiquitin signaling controls activation of nuclear factor- κ B (NF- κ B) and inflammatory responses upon engagement of pattern recognition receptors and cytokine receptors such as tumor necrosis factor (TNF) receptor 1 (TNFR1). Ubiquitin chains linked via lysine 63 (Lys63-Ub) and methionine 1 (Met1-Ub) are formed on protein substrates after receptor activation and coordinate activation of downstream kinase complexes, leading to NF- κ B-dependent transcription ¹. The linear ubiquitin chain assembly complex (LUBAC) composed of HOIP, HOIL-1, and SHARPIN assembles Met1-Ub ¹, and its function is regulated by the deubiquitinases

(DUBs) OTULIN²⁻⁴ and CYLD⁵⁻⁷ that both associate with the catalytic subunit HOIP⁵⁻⁸.

Previous studies have revealed that OTULIN exclusively hydrolyzes Met1-Ub, prevents accumulation of Met1-Ub on LUBAC components under basal conditions, and restricts ubiquitination of LUBAC substrates after receptor stimulation²⁻⁴. However, the contribution of OTULIN in regulating physiological immune responses had not been investigated.

Damgaard *et al.*⁹ now reveal that a loss-of-function mutation in *OTULIN* leads to an early-onset autoinflammatory disease termed ORAS (OTULIN related autoinflammatory syndrome). The study identifies a homozygous missense mutation in *OTULIN* (*c.815T>C*; *p.Leu272Pro*) in three affected individuals from a consanguineous family. The mutation renders OTULIN less stable and severely diminishes its DUB activity. Accordingly, isolated white blood cells from an ORAS patient exhibited elevated Met1-Ub levels as compared to healthy donor cells. ORAS patients presented with early-onset and severe systemic inflammation that can be managed with anti-TNF treatment⁹.

To dissect the mechanisms leading to ORAS, Damgaard *et al.* developed various mouse models. Mixed bone marrow chimeras were generated in which *OTULIN* was ablated in half of the bone marrow cells. This led to severe weight loss in the mice accompanied by increased neutrophil-infiltration and elevated serum levels of cytokines and chemokines. Strikingly, treating mice with anti-TNF, like in the ORAS patients, rescued most disease symptoms⁹.

Interestingly, analysis of mice where *OTULIN* was ablated in T-cells, B-cells or in myeloid cells show that ablation of *OTULIN* in B- and T-cells caused no overt phenotype whereas mice that lacked OTULIN in myeloid cells displayed enlarged

lymphoid organs and liver, had immune cell infiltration in the liver, and increased cytokine levels in serum ⁹. While this could suggest that lymphoid cells rely less on OTULIN for NF- κ B pathway regulation than myeloid cells, it would be unexpected since previous studies have found that mutations affecting LUBAC activity profoundly impacts on B- and T-cell function ¹. Instead, Damgaard *et al.* found that HOIP and SHARPIN levels were strongly reduced, presumably due to protein destabilization, in OTULIN-deficient B- and T-cells but not in the myeloid cells. This might well explain why the absence of OTULIN in B- and T-cells did not result in an overt phenotype in mice.

Analysis of signaling in OTULIN-deficient bone marrow derived macrophages (BMDM) showed, like for the ORAS patient samples, spontaneous accumulation of Met1-Ub. Additionally, OTULIN-deficient BMDMs displayed features of elevated baseline NF- κ B signaling, which was not reduced by TNF-blocking antibodies. This implies that loss of OTULIN in BMDMs causes enhanced cell-autonomous NF- κ B signaling, possibly triggered by the accumulation of Met1-Ub ⁹.

A separate recent study also identified patients with mutations in *OTULIN* ¹⁰. In addition to the L272P mutation, the study identifies two other mutations in the OTU domain. Two out of three patients respond to anti-TNF treatment with the third patient being treated with anti-IL-1 β (anakinra) and steroids. The analysis of patient samples show, in agreement with Damgaard *et al.*, increased levels of Met1-Ub and increased cytokine production relative to cells from healthy donors ¹⁰.

A number of intriguing questions arise from the findings in Damgaard *et al.* For example, how does accumulation of Met1-Ub in *OTULIN*-ablated BMDMs activate

NF- κ B independently of receptor activation? Is the Met1-Ub further modified with other linkages, for example Lys63-Ub to create a signaling platform similar to a receptor complex? It is tempting to speculate that OTULIN function under certain physiological conditions could be repressed to enable LUBAC-triggered NF- κ B signaling.

The observation that Met1-Ub accumulated in OTULIN-deficient cells rather than being disassembled by other cellular DUBs such as CYLD is intriguing because CYLD, like OTULIN, cleaves Met1-Ub and associates with HOIP⁵⁻⁷. However, OTULIN and CYLD interact with distinct LUBAC complexes in a mutually exclusive manner⁷, so a possible explanation is that CYLD only regulates ubiquitin chains conjugated by a subset of LUBAC complexes. In addition to OTULIN and CYLD, the DUB A20 has also been implicated in Met1-Ub biology. A20 does not cleave Met1-Ub but instead binds the linkage via its Zinc finger 7, leading to stabilization of Met1-Ub at the TNF receptor complex^{1, 7}. The binding contributes to A20's ability to suppress NF- κ B activity, suggestively through its recruitment to signaling complexes^{11, 12}. Taken together, there is now overwhelming evidence that regulation of LUBAC function and Met1-Ub stability by DUBs is a central regulatory mechanism in NF- κ B signaling and inflammation, which warrants further exploration into how OTULIN together with CYLD and A20 coordinate Met1-Ub signaling.

References

1 Shimizu Y, Taraborrelli L, Walczak H. Linear ubiquitination in immunity. *Immunol Rev* 2015; **266**:190-207.

- 2 Keusekotten K, Elliott PR, Glockner L *et al.* OTULIN antagonizes LUBAC signaling by specifically hydrolyzing Met1-linked polyubiquitin. *Cell* 2013; **153**:1312-1326.
- 3 Fiil BK, Damgaard RB, Wagner SA *et al.* OTULIN Restricts Met1-Linked Ubiquitination to Control Innate Immune Signaling. *Molecular cell* 2013; **50**:818-830.
- 4 Rivkin E, Almeida SM, Ceccarelli DF *et al.* The linear ubiquitin-specific deubiquitinase gumby regulates angiogenesis. *Nature* 2013; **498**:318-324.
- 5 Takiuchi T, Nakagawa T, Tamiya H *et al.* Suppression of LUBAC-mediated linear ubiquitination by a specific interaction between LUBAC and the deubiquitinases CYLD and OTULIN. *Genes to cells* 2014; **19**:254-272.
- 6 Hrdinka M, Fiil BK, Zucca M *et al.* CYLD Limits Lys63- and Met1-Linked Ubiquitin at Receptor Complexes to Regulate Innate Immune Signaling. *Cell reports* 2016; **14**:2846-2858.
- 7 Draber P, Kupka S, Reichert M *et al.* LUBAC-Recruited CYLD and A20 Regulate Gene Activation and Cell Death by Exerting Opposing Effects on Linear Ubiquitin in Signaling Complexes. *Cell reports* 2015; **13**:2258-2272.
- 8 Elliott PR, Nielsen SV, Marco-Casanova P *et al.* Molecular basis and regulation of OTULIN-LUBAC interaction. *Mol Cell* 2014; **54**:335-348.
- 9 Damgaard RB, Walker JA, Marco-Casanova P *et al.* The Deubiquitinase OTULIN Is an Essential Negative Regulator of Inflammation and Autoimmunity. *Cell* 2016; **166**:1215-1230 e1220.
- 10 Zhou Q, Yu X, Demirkaya E *et al.* Biallelic hypomorphic mutations in a linear deubiquitinase define otulipenia, an early-onset autoinflammatory disease. *Proc Natl Acad Sci U S A* 2016.

11 Verhelst K, Carpentier I, Kreike M *et al.* A20 inhibits LUBAC-mediated NF-kappaB activation by binding linear polyubiquitin chains via its zinc finger 7. *Embo J* 2012; **31**:3845-3855.

12 Tokunaga F, Nishimasu H, Ishitani R *et al.* Specific recognition of linear polyubiquitin by A20 zinc finger 7 is involved in NF-kappaB regulation. *Embo J* 2012; **31**:3856-3870.

Figure Legend.

Figure 1. Activation of immune receptors triggers assembly of Met1-Ub by LUBAC that facilitate inflammation and under normal conditions is counter-balanced by the deubiquitinase OTULIN. Individuals with a loss-of-function mutation in OTULIN (L272P) or mice where OTULIN is deleted in myeloid cells accumulate Met1-Ub, possibly independently of receptor activation, and develop a severe autoinflammatory condition termed OTULIN related autoinflammatory syndrome (ORAS). Significantly, ORAS can be alleviated by the inhibition of TNF.