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## RESEARCH HIGHLIGHT

# HOIL1 cleavage by MALT1, the knives are out

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The paracaspase MALT1 functions as a bifunctional regulator of lymphocyte activation following the engagement of antigen receptors. First, MALT1 scaffolds the CARMA1-BCL10-MALT1 (CBM) signaling complex in charge of activating the NF- $\kappa$ B transcription factor. Second, MALT1 proteolytic activity governs NF- $\kappa$ B fine-tuning and the homeostasis of the immune system. MALT1 is also constitutively activated in the activated B-cell like (ABC) subset of diffuse large B-cell lymphoma (DLBCL), and the discovery that its chemical inhibition is toxic has opened new perspectives of treatment. Yet, the nature of MALT1 substrates continues to be elucidated. Herein, we review the recent identification of the linear ubiquitin assembly chain complex (LUBAC) element HOIL1 as a new substrate for MALT1 in lymphocytes and lymphoma. We discuss how this processing may affect NF- $\kappa$ B signaling and impact on lymphocyte homeostasis.

**Keywords:** Lymphocyte; MALT1; LUBAC; Signaling; Lymphoma; NF- $\kappa$ B

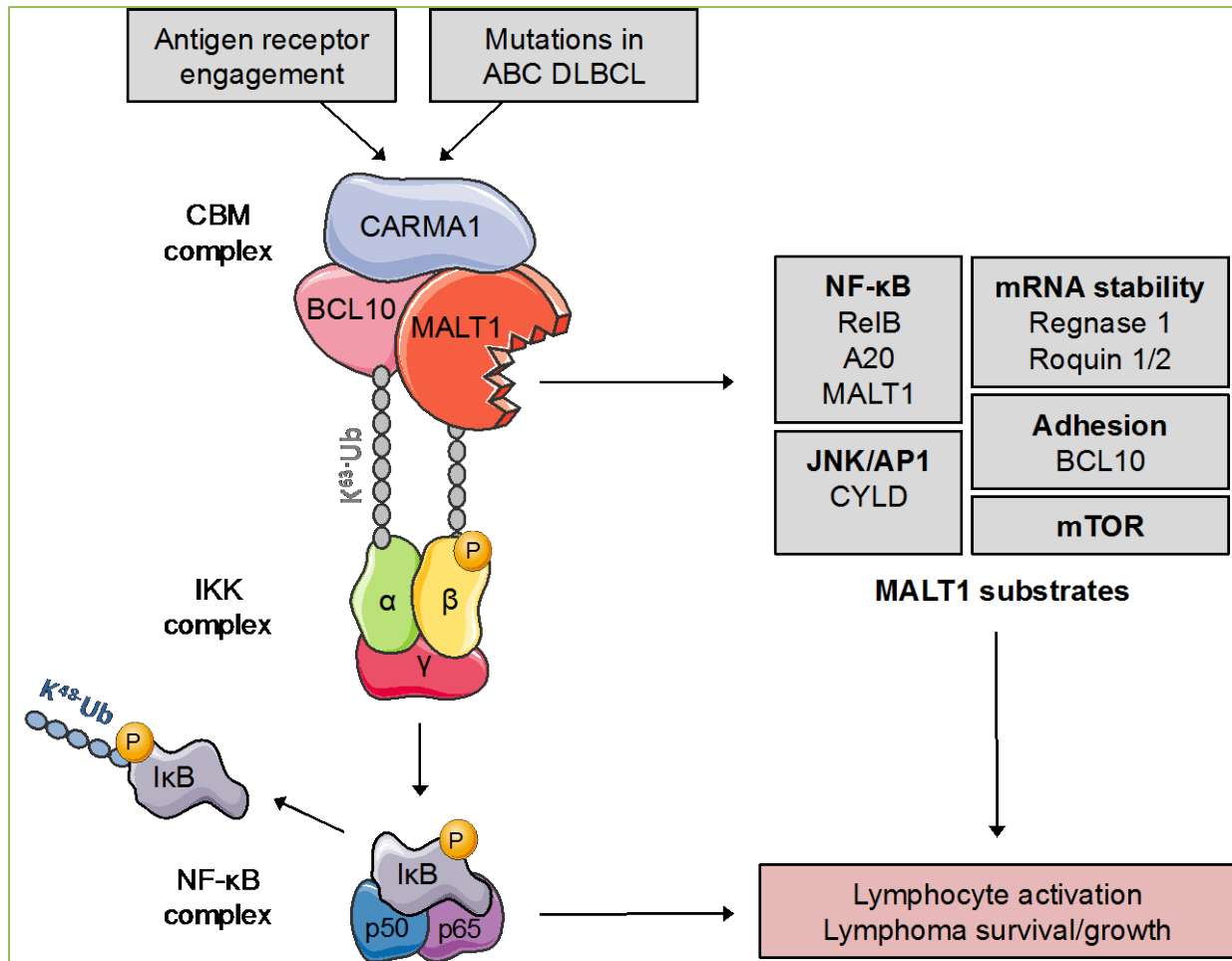
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The activation of the transcription factor NF- $\kappa$ B is a pivotal event in lymphocyte activation and in cellular homeostasis in the immune system. NF- $\kappa$ B heterodimers are normally tethered to their cognate inhibitors, I $\kappa$ Bs, in the cytosol. The engagement of antigen receptors in B and T lymphocytes assembles a large signaling complex composed of CARMA1, BCL10, and MALT1 (the CBM complex) in charge of activating the I $\kappa$ B kinase (IKK) complex<sup>[1]</sup>. IKK-mediated phosphorylation and subsequent proteasomal degradation of I $\kappa$ Bs causes NF- $\kappa$ B to shuttle to the nucleus and exert its transcriptional activity (Figure 1)<sup>[1]</sup>. Piracy of NF- $\kappa$ B signaling pathway to sustain its activity is a mechanism deployed by the Activated B-cell like (ABC) subtype of diffuse large B-Cell lymphoma (DLBCL) to

counteract cell death and promote unlimited growth<sup>[2]</sup>. This aberrant activation of NF- $\kappa$ B results from somatic mutations in *CD79B*, *CARMA1* (also called *CARD11*), *MYD88*, and *A20* (also called *TNFAIP3*) genes<sup>[2]</sup>. The discovery that ABC DLBCL hijack the CBM complex to maintain the constitutive activation of NF- $\kappa$ B has opened new perspectives for selective treatments.

In addition to its scaffold function during NF- $\kappa$ B activation, the paracaspase activity of MALT1 finely tunes immune responses and allows ABC DLBCL survival<sup>[1]</sup>. The mono-ubiquitination on MALT1 Lysine residue 644 is required to unleash its catalytic activity upon antigen receptor engagement<sup>[3]</sup>. Known substrates include negative



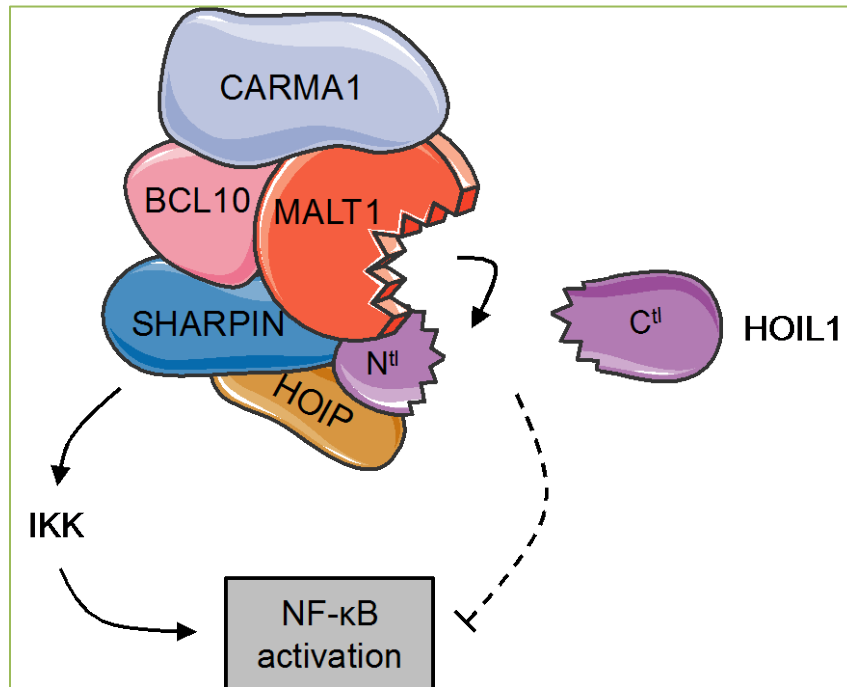
**Figure 1. MALT1 exerts dual roles during lymphocytes activation and lymphoma survival.** MALT1 scaffolds the CARMA1-BCL10-MALT1 (CBM) complex to promote NF-κB activation. MALT1 also cleaves multiple substrates to ensure the fine-tuning of the immune response.

regulators of NF-κB (A20, RelB and MALT1), adhesion (BCL10), JNK (CYLD), and mTORC1, as well as mRNA stability factors (Regnase-1, Roquin-1/2) (Figure 1) [1]. Importantly, MALT1 is aberrantly activated in ABC DLBCL and its chemical inhibition is toxic [4-7]. Nevertheless, the inactivation of MALT1 enzyme in mice induces a lethal multi-organ inflammatory syndrome, resulting from aberrant production of interferon gamma (IFN $\gamma$ ) by effector lymphocytes [8-10]. MALT1 protease also governs the development of innate-like B cells and regulatory T cells [8-10]. Last, mature lymphocytes display decreased antigen receptor-mediated proliferation, diminished IL-2 and TNF $\alpha$  production, and reduced Th17 differentiation [8-10]. As a consequence, MALT1 inactivation protects against experimental autoimmune encephalomyelitis (EAE) [8, 10].

The striking phenotype of protease-dead MALT1 mice urges us to establish the landscape of MALT1 substrates [8-10]. Three independent groups, including ours, now report the identification of the E3 ligase HOIL1 as a new substrate

of MALT1 [11-13]. HOIL1 forms together with E3 ligase HOIP and the SHANK-containing protein SHARPIN the LUBAC (Linear Ubiquitin chain Assembly Complex). The LUBAC catalyzes "head-to-tail" poly-ubiquitin chains and participates in multiple signaling pathways converging on NF-κB [14]. In that view, the LUBAC was recently found to be an essential part of the CBM needed for IKK activation in lymphocytes and in ABC DLBCL [15-17]. Although HOIP and SHARPIN are instrumental to convey NF-κB signaling, the role of HOIL1 has remained obscure [15].

MALT1 is a cysteine protease that cleaves its substrates after an arginine residue embedded in a consensus S/PR↓G domain [18]. Because MALT1 is activated within the CBM microenvironment, we reasoned that putative substrates transit through this signalosome, and therefore performed an *in silico* analysis of known partners in the literature. This led to the identification of the LQPR<sup>165</sup>G motif in HOIL1 sequence [11]. The overexpression of HOIL1 in HEK293T cells together with BCL10 and MALT1 led to HOIL1



**Figure 2. Working model for HOIL1 function in lymphocytes.** HOIL1 negatively regulates antigen receptor-mediated NF-κB activation unless cleaved by MALT1. While HOIL1<sup>C<sup>tl</sup></sup> is released from the LUBAC and CBM complexes, HOIL1<sup>N<sup>tl</sup></sup> maintains the LUBAC architecture and allows NF-κB activation.

cleavage, which was abolished when the R<sup>165</sup> residue was substituted with an alanine or a glycine<sup>[11]</sup>. We further found that HOIL1 was processed by MALT1 after R<sup>165</sup> following antigen receptor engagement in the human lymphoblastoid T cell line Jurkat as well as in primary mouse T lymphocytes. This was however not the case when cells were treated with TNFα, which activates NF-κB independently of the CBM complex. Importantly, HOIL1 was constitutively processed by MALT1 in ABC DLBCL cell lines, which exhibit an aberrant activity of the protease<sup>[11]</sup>. Additional work will be required to define the impact of this cleavage on the biology of this aggressive lymphoma. Concomitantly to this work, Elton *et al* independently established that MALT1 trims HOIL1 after the R<sup>165</sup> residue in Jurkat and primary T lymphocytes. They further demonstrated that overexpressing the oncogenic MALT1-AP1, which corresponds to the in frame fusion of NH<sub>2</sub>-terminal part of API2 with the COOH-terminal domain of MALT1, also led to HOIL1 proteolysis<sup>[12]</sup>. Klein and coworkers also reached the same conclusion by developing an elegant 10-plex Tandem Mass Tag TAILS N-terminal peptide proteomics approach using cells derived from a patient with a genetic MALT1 deficiency<sup>[13]</sup>. Overall, these data establish HOIL1 as a *bona fide* substrate of MALT1.

To next evaluate the impact of HOIL1 cleavage on NF-κB activation, we introduced a MALT1-resistant version of

HOIL1 (HOIL1<sup>R165G</sup>) in Jurkat cells. This hampered the activation of NF-κB and the secretion of downstream interleukin-2 in response to antigen receptor stimulation<sup>[11]</sup>. Although the molecular mechanism by which HOIL1 marshals NF-κB remains elusive, interfering with HOIL1 cleavage did not alter IKK activation<sup>[11]</sup>. This suggests that HOIL1 belongs, together with A20 and RelB, to a group of proteins that lessens NF-κB activation when left uncleaved by MALT1.

The function of the two HOIL1 fragments resulting from MALT1 enzyme remains unclear as apparently conflicting findings were obtained<sup>[11-13]</sup>. HOIL1<sup>N<sup>ter</sup></sup> contains an ubiquitin-like (UBL) segment, which maintains the LUBAC stability and allows the LUBAC to drive NF-κB activation<sup>[19]</sup>. In line with this, the overexpression of HOIL1<sup>N<sup>ter</sup></sup> together with HOIP promotes NF-κB activation<sup>[12]</sup>. By contrast, HOIL1<sup>C<sup>ter</sup></sup> essentially bears the E3 ligase activity and counteracts HOIL1<sup>N<sup>ter</sup></sup>-mediated NF-κB activation when overexpressed in HEK293T cells together with the other LUBAC subunits<sup>[12, 13]</sup>. Klein *et al* further propose that HOIL1 proteolysis destabilizes the LUBAC, thereby decreasing linear ubiquitination and NF-κB activation<sup>[13]</sup>. This is however challenged by the finding that HOIL1 is constitutively and massively cleaved in lysates from ABC DLBCL cells<sup>[11]</sup>. Those cell lines display aberrant activation of NF-κB and constitutive linear ubiquitination of NEMO

and BCL10<sup>[16, 17]</sup>. Further supporting an active role for the LUBAC in ABC DLBCL despite HOIL1 processing, the silencing of HOIP and SHARPIN is lethal<sup>[15, 17]</sup>. In keeping with this, HOIP and SHARPIN levels remained unchanged in cells treated with the MALT1 tetrapeptide inhibitor z-VRPR.fmk<sup>[11]</sup>. Our findings support a model in which MALT1 only alleviates HOIL1 negative grip on NF- $\kappa$ B while maintaining the LUBAC function via HOIL1<sup>Nter</sup> (Figure 2). Of note, HOIL1<sup>Cter</sup> is not part of the CBM or LUBAC complexes, and is released into the cytosol (Ref<sup>[11]</sup> and our unpublished results). Lastly, the enforced expression of HOIL1<sup>Nter</sup> or HOIL1<sup>Cter</sup> had no overt effect on NF- $\kappa$ B activation<sup>[11]</sup>, reinforcing the idea that HOIL1 as a negative regulator of NF- $\kappa$ B cleaved by MALT1.

In conclusion, three studies identified the LUBAC subunit HOIL1 as a new substrate of MALT1 during antigen receptor signaling and in ABC DLBCL cells. We propose that HOIL1 functions as a negative regulator of NF- $\kappa$ B inactivated by MALT1 proteolysis. Further investigations are needed to delineate how HOIL1 thwarts NF- $\kappa$ B signaling when uncleaved. Because HOIL1 has been shown to catalyze degradative Lys-48 (K<sup>48</sup>)-linked ubiquitination<sup>[20]</sup>, it is tempting to speculate that HOIL1 promotes proteasomal degradation of substrates and that MALT1 cleavage counteracts HOIL1 enzyme activity. Identifying those HOIL1 substrates may open new breaches to modulate lymphocyte activation and restore vulnerability in some lymphoma.

### Conflicting interests

The authors have declared that no conflict of interests exist.

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### Author contributions

T.D. and N.B. wrote and approved the final manuscript.

### Abbreviation

ABC DLBCL: activated B-cell like diffuse large B-cell lymphoma; CBM: CARMA1-BCL10-MALT1; EAE: experimental autoimmune encephalomyelitis; IFN $\gamma$ : interferon gamma; IKK: I $\kappa$ B kinase complex; NF- $\kappa$ B: nuclear factor  $\kappa$ B; UBL: ubiquitin-like.

### References

- Juillard M, and Thome M. Role of the CARMA1/BCL10/MALT1 complex in lymphoid malignancies. *Curr Opin Hematol* 2016; 23, 402-409.
- Shaffer AL, 3rd, Young RM, and Staudt LM. Pathogenesis of human B cell lymphomas. *Annu Rev Immunol* 2012; 30, 565-610.
- Pelzer C, Cabalzar K, Wolf A, Gonzalez M, Lenz G, and Thome M. The protease activity of the paracaspase MALT1 is controlled by monoubiquitination. *Nat Immunol* 2013; 14, 337-345.
- Ferch U, Kloob B, Gewies A, Pfander V, Duwel M, Peschel C, et al. Inhibition of MALT1 protease activity is selectively toxic for activated B cell-like diffuse large B cell lymphoma cells. *J Exp Med* 2009; 206, 2313-2320.
- Fontan L, Yang C, Kabaleeswaran V, Volpon L, Osborne MJ, Beltran E, et al. MALT1 small molecule inhibitors specifically suppress ABC-DLBCL in vitro and in vivo. *Cancer Cell* 2012; 22, 812-824.
- Hailfinger S, Lenz G, Ngo V, Posvitz-Fejfar A, Rebeaud F, Guzzardi M, et al. Essential role of MALT1 protease activity in activated B cell-like diffuse large B-cell lymphoma. *Proc Natl Acad Sci U S A* 2009; 106, 19946-19951.
- Nagel D, Spranger S, Vincendeau M, Grau M, Raffegerst S, Kloob B, et al. Pharmacologic inhibition of MALT1 protease by phenothiazines as a therapeutic approach for the treatment of aggressive ABC-DLBCL. *Cancer Cell* 2012; 22, 825-837.
- Bornancin F, Renner F, Touil R, Sic H, Kolb Y, Touil-Allaoui I, et al. Deficiency of MALT1 paracaspase activity results in unbalanced regulatory and effector T and B cell responses leading to multiorgan inflammation. *J Immunol* 2015; 194, 3723-3734.
- Gewies A, Gorka O, Bergmann H, Pechloff K, Petermann F, Jeltsch KM, et al. Uncoupling Malt1 threshold function from paracaspase activity results in destructive autoimmune inflammation. *Cell Rep* 2014; 9, 1292-1305.
- Jaworski M, Marsland BJ, Gehrig J, Held W, Favre S, Luther SA, et al. Malt1 protease inactivation efficiently dampens immune responses but causes spontaneous autoimmunity. *EMBO J* 2014; 33, 2765-2781.
- Douanne T, Gavard J, and Bidere N. The paracaspase MALT1 cleaves the LUBAC subunit HOIL1 during antigen receptor signaling. *J Cell Sci* 2016; 129, 1775-1780.
- Elton L, Carpentier I, Staal J, Driege Y, Haegman M, and Beyaert R. MALT1 cleaves the E3 ubiquitin ligase HOIL-1 in activated T cells, generating a dominant negative inhibitor of LUBAC-induced NF-kappaB signaling. *The FEBS journal* 2015; 283: 403-412.
- Klein T, Fung SY, Renner F, Blank MA, Dufour A, Kang S, et al. The paracaspase MALT1 cleaves HOIL1 reducing linear ubiquitination by LUBAC to dampen lymphocyte NF-kappaB signalling. *Nature communications* 2015; 6, 8777.
- Iwai K, Fujita H, and Sasaki Y. Linear ubiquitin chains: NF-kappaB signalling, cell death and beyond. *Nat Rev Mol Cell Biol* 2014; 15, 503-508.
- Dubois SM, Alexia C, Wu Y, Leclair HM, Leveau C, Schol E, et al. A catalytic-independent role for the LUBAC in NF-kappaB activation upon antigen receptor engagement and in lymphoma cells. *Blood* 2014; 123, 2199-2203.

16. Yang Y, Kelly P, Shaffer AL, 3rd, Schmitz R, Yoo HM, Liu X, *et al.* Targeting Non-proteolytic Protein Ubiquitination for the Treatment of Diffuse Large B Cell Lymphoma. *Cancer Cell* 2016; 29, 494-507.
17. Yang Y, Schmitz R, Mitala J, Whiting A, Xiao W, Ceribelli M, *et al.* Essential role of the linear ubiquitin chain assembly complex in lymphoma revealed by rare germline polymorphisms. *Cancer discovery* 2014; 4, 480-493.
18. Coornaert B, Baens M, Heyninck K, Bekaert T, Haegman M, Staal J, *et al.* T cell antigen receptor stimulation induces MALT1 paracaspase-mediated cleavage of the NF-kappaB inhibitor A20. *Nat Immunol* 2008; 9, 263-271.
19. Tokunaga F, Sakata S, Saeki Y, Satomi Y, Kirisako T, Kamei K, *et al.* Involvement of linear polyubiquitylation of NEMO in NF-kappaB activation. *Nature cell biology* 2009; 11, 123-132.
20. Elton L, Carpentier I, Verhelst K, Staal J, and Beyaert R. The multifaceted role of the E3 ubiquitin ligase HOIL-1: beyond linear ubiquitination. *Immunol Rev* 2015; 266, 208-221.