Autophagy 10:6, 1146–1147; June 2014; © 2014 Landes Bioscience

Autophagy side of MB21D1/cGAS DNA sensor

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The MB21D1/cGAS (Mab-21 domain-containing 1/cyclic GMP-AMP [cGAMP] synthetase), acts as an intracellular pattern recognition receptor (PPR) to sense cytosolic pathogen DNAs and subsequently generates the second messenger cGAMP to initiate the TMEM173/STING pathway for interferon (IFN) production. Intriguingly, we have recently demonstrated crosstalk between the intracellular DNA sensing pathway and autophagy machinery by demonstrating a direct interaction between the MB21D1 DNA sensor and the BECN1/Beclin 1 autophagy protein. This interaction not only suppresses MB21D1 enzymatic activity to halt cGAMP production, but also enhances the autophagy-mediated degradation of cytosolic microbial DNAs. This demonstrates that MB21D1 is the molecular link between the intracellular DNA sensing pathway and the autophagy pathway, ultimately developing well-balanced immune responses against pathogens.

Keywords: cGAS, Beclin 1, autophagy, interferon

Submitted: 03/06/2014

Revised: 03/31/2014

Accepted: 04/03/2014

Published Online: 04/24/2014

http://dx.doi.org/10.4161/auto.28769

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Punctum to: Liang Q, Seo GJ, Choi YJ, Kwak MJ, Ge J, Rodgers MA, Shi M, Leslie BJ, Hopfner KP, Ha T, et al. Crosstalk between the cGAS DNA sensor and Beclin-1 autophagy protein shapes innate antimicrobial immune responses. Cell Host Microbe 2014; 15:228-38; PMID:24528868; http://dx.doi.org/10.1016/j.chom.2014.01.009

Innate immune detection of nucleic acids is a critical component of host responses to microbial infection. Upon microbial infection, intracellular pattern recognition receptors (PRRs) can detect microbes' conserved patterns, such as their specific nucleic acids or chemicals, to activate signaling cascades, thereby triggering type I IFN-mediated defense mechanisms. MB21D1 acts as an intracellular PRR to sense cytosolic pathogen DNAs and subsequently generates the second messenger cGAMP that can bind the downstream signaling molecule TMEM173, leading to the production of type I IFNs. Indeed, MB21D1-deficient mice fail to produce IFNs and other cytokines in response to DNA transfection or DNA virus infection and are more susceptible to lethal herpes simplex virus infection than wild-type mice. MB21D1 possesses a remarkable structural similarity to OAS1 (2′-5′ oligoadenylate synthetase 1, 40/46 kDa), an antiviral cytosolic double-stranded RNA sensor, but contains a unique zinc thumb that recognizes B-form double-stranded DNA. These suggest OAS1-mediated dsRNA and MB21D1-mediated dsDNA innate immune sensing machinery may share a molecular mechanism.

Autophagy is an important homeostatic mechanism involving the formation of double-membrane vesicles, called autophagosomes, which sequester damaged organelles and protein aggregates in the cytoplasm for degradation. Conserved from yeast to humans, autophagy takes place through a series of steps that include vesicle initiation, nucleation, and elongation, followed by vesicle fusion with lysosomes for cargo degradation. In addition to this basic catabolic role, autophagy plays a central role in host defense response to pathogens by promoting the elimination of invading microbes that enter into the cytosol. Watson et al. (2012) first reported that extracellular *M. tuberculosis* DNA triggers autophagy and demonstrated a link between cytosolic DNA and autophagy. However, the underlying mechanisms remain elusive. Our recent work has identified MB21D1 as the sensor for dsDNA-mediated autophagy by interacting with BECN1. MB21D1 contains a highly positively charged N-terminal domain and a central

nucleotidyl transferase (NTase) domain that partially overlaps with a male abnormal 21 C-terminal domain. Upon DNA sensing, MB21D1 forms predominantly cytoplasmic punctate structures and recruits BECN1 into this compartment via a direct interaction. Interestingly, the DNA binding activity, but not the enzymatic activity, of MB21D1 is required for the BECN1 interaction and cytoplasmic punctate formation. In vitro reconstitution and in vivo functional assays demonstrate that BECN1 interaction suppresses the NTase activity of MB21D1, decreasing cGAMP synthesis and thereby decreasing IFNB1 production. This indicates that BECN1 interaction is a negative feedback regulatory mechanism of MB21D1-mediated IFN production.

Extensive studies have demonstrated that BECN1 plays pivotal roles in autophagy induction and autophagosome maturation by forming various complexes with its positive (ATG14 and UVRAG) or negative (BCL2 and KIAA0226/Rubicon) regulators. Besides the inhibitory role of BECN1 interaction in MB21D1 function, we also showed that the interaction between MB21D1 and BECN1 is important for triggering the release of KIAA0226 from BECN1 autophagy complexes. KIAA0226 interacts with BECN1, UVRAG, and PIK3C3 and functions as a negative regulator for PIK3C3 to inhibit autophagy. Under normal conditions, most BECN1 is present within KIAA0226-containing complexes; however, upon dsDNA transfection, BECN1 moves from KIAA0226-complexes to MB21D1-complexes. Detailed mapping showed that since both KIAA0226 and MB21D1 bind the coiled-coil domain of BECN1, MB21D1 competes with KIAA0226 for BECN1 binding upon dsDNA stimulation, promoting the dissociation of KIAA0226 from BECN1 complexes and thereby, leading to PIK3C3 kinase activation and autophagy induction to remove cytosolic pathogen DNAs. Surprisingly, we also found that the second messenger cGAMP triggered autophagy in a TMEM173-dependent, but MB21D1-independent, manner, which is consistent with the previous finding that another cyclic dinucleotide, c-di-GMP, from bacteria triggers TMEM173-dependent autophagy. These results indicate that at the early stage of infection, MB21D1 senses microbial dsDNAs and subsequently generates the second messenger cGAMP that can bind the downstream signaling molecule TMEM173, leading to the production of type I IFNs. At the late stage of the host immune response, MB21D1 may shuttle between the TMEM173-mediated IFN pathway and the BECN1-mediated autophagy pathway to elicit IFN production, while inducing autophagy-mediated microbial DNA degradation to avoid persistent immune stimulation.

This study is the first to establish the dual role of MB21D1 in both IFN signaling and autophagy, which also poses a number of questions. First, how are MB21D1 and BECN1 recruited to cytosolic punctate structures upon dsDNA stimulation? Since BECN1 undergoes various posttranslational modifications such as phosphorylation, ubiquitination, and acetylation during autophagy conditions, it will be interesting to examine whether BECN1 and/or MB21D1 undergoes certain modifications upon stimulation. Second, how does MB21D1 specifically release the negative regulator KIAA0226 from BECN1 complexes without affecting other components such as PIK3C3 and ATG14? Since PIK3C3 binds to the evolutionarily conserved C-terminal domain of BECN1, MB21D1 interaction may not affect the PIK3C3-BECN1 interaction. The BECN1 CCD can be subdivided into 4 fragments (CCD1-4) and both MB21D1 and KIAA0226 preferentially bind the first 2 fragments (CCD1-2). One possibility is that ATG14 may preferentially bind the last 2 fragments (CCD3-4) of BECN1, avoiding competition with MB21D1. Further structural analysis will be helpful for understanding the relationship between the MB21D1 and BECN1 complex in detail. Third, how does cGAMP induce autophagy in a TMEM173-dependent manner? Knono et al. (2013) reported that cGAMP induces the dephosphorylation of the ULK1 autophagy kinase, which subsequently phosphorylates TMEM173 and blocks its function, initiating negative-feedback control of the TMEM173 pathway. However, dephosphorylation is an inhibitory mechanism of the ULK1-mediated autophagy pathway. It is possible that the cGAMP-bound TMEM173 may directly or indirectly target autophagy proteins that subsequently turn on autophagy. Finally, are there any viral or bacterial factors that modulate MB21D1-mediated host immunity? Since MB21D1 plays a critical role in both IFN- and autophagy-mediated antimicrobial actions, pathogens should overcome MB21D1 activity to establish their life cycles. Nevertheless, these studies suggest that the MB21D1-TMEM173 pathway has at least 2 independent, negative-feedback regulatory mechanisms such as the BECN1-mediated suppression of MB21D1 activity and the ULK1-mediated inhibition of TMEM173 activity, which ultimately keeps the host immune system in check. Thus, halting PRR signaling is as important as initiating it.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.