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EDITORIALS: CELL CYCLE FEATURES

SPATA2: New insights into the assembly of the TNFR signaling complex

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Non-degradative ubiquitylation represents a pivotal posttranscriptional modification (PTM) for signaling by the inflammatory cytokine TNF. K63- and M1-linked ubiquitylation of RIPK1, NEMO, cIAPs, TRADD, and TNFR1 promotes the assembly and the stabilization of the TNF-receptor signaling complex (TNF-RSC). In this context, the IAPs and the LUBAC complex have been identified as the main mediators of K63- and M1-linked ubiquitylation, respectively.

Ubiquitylation, like other PTMs, is reversible. Enzymes such as CYLD, A20 or OTULIN have been shown to deubiquitylate a number of components of the TNF-RSC, and these deubiquitinases (DUBs) exhibit specificity for different ubiquitin linkages. The DUB CYLD is unique, by being specific for both K63- and M1-linkages. By proteolytic cleavage of those bondings, CYLD deubiquitinates TNF-RSC components, resulting in TNF-RSC destabilization, and thus attenuating the signal triggered by TNFR stimulation. In order to perform its job, CYLD is recruited to the TNF-RSC. Importantly, it was recently shown that CYLD is recruited to the TNF-RSC along with the M1- ubiquitin ligase complex LUBAC, while OTULIN, another M1-specific DUB and binding partner of HOIP, was not.[1]

Our recent study provides new insight as to how the recruitment of CYLD to the TNF-RSC is organized.[2] By performing a SILAC mass-spectrometry screen for interaction partners of CYLD, we identified the protein spermatogenesis associated protein 2, SPATA2, which stood out in our screen with a high enrichment score. This protein had previously been reported to be involved in spermatogenesis, while other reports had discussed the involvement of the *spata2* locus in psoriasis. We re-assessed the interaction by co-immunoprecipitation experiments and found that SPATA2 interacts with its N-terminal PUB domain with the C-terminus of CYLD, which comprises the ubiquitin specific protease (USP) activity domain. As the recruitment of CYLD to the TNF-RSC had been shown to require the LUBAC component HOIP, we tested the possibility that SPATA2 interacts with HOIP as well. This was indeed the case, as HOIP interacted with SPATA2 via its PUB domain, while a PUB-interacting motif (PIM) in SPATA2, which we identified, was required for the interaction with HOIP. Thus, our data suggested that SPATA2 is the protein, which mediates the interaction between CYLD and HOIP. Consistent with these interaction studies, SPATA2 was recruited with similar kinetics, as were CYLD and HOIP, to the TNF-RSC, upon TNF stimulation.

Furthermore, when cells lacking SPATA2 were stimulated with TNF, the recruitment of HOIP was preserved, while CYLD was absent from the TNF-RSC. These findings, along with data from another study, strongly suggests SPATA2 to be the bridging factor for the CYLD-HOIP interaction, and thereby for the recruitment of CYLD to the TNF-RSC.[3]

The fact that SPATA2 interacted with the USP domain of CYLD let us wonder whether the enzyme activity of CYLD itself is affected by the interaction with SPATA2. Intriguingly, we observed that CYLD exhibited an increased enzyme activity for cleavage of K63- and M1-linked di-ubiquitin substrate, when SPATA2 was simultaneously overexpressed.

In line with these data, cells lacking SPATA2 exhibited increased M1 ubiquitylation at the TNF-RSC. This suggested that the signaling downstream of the TNFR1 would be affected in SPATA2$^{-/-}$cells, and indeed, SPATA2 ko cells exhibited both enhanced NF-κB and MAPK signaling after stimulation with TNF. This indicated that SPATA2, just as CYLD, acts as a negative regulator of these signaling pathways. It is quite likely that SPATA2 also influences signaling pathways downstream of NODs and TLRs, making SPATA2 a novel important player in the field of innate immunity.

In addition, an important question was whether SPATA2 also affects the death-inducing activity of TNF. TNF-induced death signaling requires the absence of pro-survival signals such as IAP- or LUBAC-mediated ubiquitylation, or the absence of TAK1 activity,[4-6] allowing the formation of the TNFR complex II. By analyzing TAK1$^{-/-}$ cells, which we stimulated with TNF, we found that the additional absence of SPATA2 largely attenuated the formation of complex II. Consistently, TAK1$^{-/-}$/SPATA2$^{-/-}$ cells showed very low caspase activity and reduced apoptosis, as compared to cells lacking TAK1 only, which underwent rapid,

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RIPK1-dependent apoptosis. Thus, our study shows that SPATA2 is required for TNF induced, RIPK1-dependent apoptosis in cells permissive for this signaling pathway, and it will be important to explore whether SPATA2 is part of complex II.[2]

What are the biological implications? A number of different studies on CYLD$^{-/-}$ mice reported male infertility, defects in T-cell development, hyper-responsive T-and B- cells, as well as an hyper-activation of macrophages in absence of CYLD.[7] It will be very interesting to see whether these findings, or at least some of them, are reflected in SPATA2$^{-/-}$ animals.

SPATA2 was initially identified in testes, and shown to be highly expressed in Sertoli cells. In fact, we could demonstrate that SPATA2 reduced NF-κB and MAPK activation by TNF in 15P-1, a Sertoli cell line. It is tempting to speculate that SPATA2 plays a role to dampen cytokine secretion by Sertoli cells, in order to attenuate potentially damaging inflammatory responses. The analysis of the fertility of SPATA2$^{-/-}$ males will very likely shed light on the requirement of SPATA2 to preserve the integrity of male reproduction.

Taken together, with SPATA2, a novel essential component of the TNF-RSC was identified and further research will help us to complete the understanding of its role for innate immunity and cell death signaling pathways.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

References

[1] Draber P, Kupka S, Reichert M, Draberova H, Lafont E, de Miguel D, Spilgies L, Surinova S, Taraborrelli L, Hartwig T, 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; 13:2258-72; PMID:26670046; http://dx.doi.org/10.1016/j.celrep.2015.11.009

[2] Schlicher L, Wissler M, Preiss F, Brauns-Schubert P, Jakob C, Dumit V, Borner C, Dengjel J, Maurer U. SPATA2 promotes CYLD activity and regulates TNF-induced NF-κB signaling and cell death. EMBO Rep 2016; PMID:27458237; http://dx.doi.org/10.15252/embr.201642592

[3] Wagner SA, Satpathy S, Beli P, Choudhary C. SPATA2 links CYLD to the TNF-α receptor signaling complex and modulates the receptor signaling outcomes. EMBO J 2016; 35:1868–84; http://dx.doi.org/10.15252/embj.201694300

[4] Bertrand MJM, Milutinovic S, Dickson KM, Ho WC, Boudreault A, Durkin J, Gillard JW, Jaquith JB, Morris SJ, Barker PA. cIAP1 and cIAP2 facilitate cancer cell survival by functioning as E3 ligases that promote RIP1 ubiquitination. Mol Cell 2008; 30:689-700; PMID:18570872; http://dx.doi.org/10.1016/j.molcel.2008.05.014

[5] Peltzer N, Rieser E, Taraborrelli L, Draber P, Darding M, Pernaute B, Shimizu Y, Sarr A, Draberova H, Montinaro A, et al. HOIP deficiency causes embryonic lethality by aberrant TNFR1-mediated endothelial cell death. Cell Reports 2014; 9:153-65; PMID:25284787; http://dx.doi.org/10.1016/j.celrep.2014.08.066

[6] Vanlangenakker N, Vanden Berghe T, Bogaert P, Laukens B, Zobel K, Deshayes K, Vucic D, Fulda S, Vandenabeele P, Bertrand MJM. cIAP1 and TAK1 protect cells from TNF-induced necrosis by preventing RIP1/RIP3-dependent reactive oxygen species production. Cell Death Differ 2011; 18:656-65; PMID:21052097; http://dx.doi.org/10.1038/cdd.2010.138

[7] Sun SC. CYLD: a tumor suppressor deubiquitinase regulating NF-kappaB activation and diverse biological processes. Cell Death Differ 2010; 17:25-34; PMID:19373246; http://dx.doi.org/10.1038/cdd.2009.43