IN THE SPOTLIGHT

Germline Polymorphisms in RNF31 Regulate Linear Ubiquitination and Oncogenic Signaling

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Summary: A new study proposes that the cancerous behavior of B cells in a subtype of diffuse large B-cell lymphoma is caused by excessive activity of the linear ubiquitin chain assembly complex (LUBAC) complex, which underlies abnormal NF-κB signaling. Two rare germline single-nucleotide polymorphisms in the RNF31 gene have been identified as being responsible. The use of a small inhibiting peptide may downregulate the abnormal LUBAC activity and counteract neoplastic cell growth. Cancer Discov; 4(4); 394–6. ©2014 AACR.

See related article by Yang et al., p. 480 (3).

Single-nucleotide polymorphisms (SNP) add the spice to the genomic soup (1). In addition to factors such as environmental and societal influences, these small variations in the DNA sequence are important drivers for individuality and diversity (2). They also affect our susceptibility to diseases and modulate response to therapy. Many human diseases result from SNPs, and SNPs have been extremely useful as markers in the hunt for disease-associated genes in genome-wide association studies. In addition, the analysis of SNPs and the availability of tailored genotyping platforms have also had an enormous impact on personalized medicine and may well revolutionize the field. However, not all polymorphisms in the human genome have been discovered nor their relevance understood; in this respect, the genome is still a treasure trove of discoveries.

The feelings of Yang and colleagues (3) must have been similar when they realized that two missense germline SNPs in exon 10 of the RNF31 gene are enriched in patients with the activated B cell–like (ABC) subtype of diffuse large B-cell lymphoma (DLBCL), as they describe in this issue of Cancer Discovery.

RNF31 is a RING finger protein, also known as HOIL-1 interacting protein [HOIP; belonging to the RING–IBR–RING (RBRR) protein family (4, 5)]. The interaction of RNF31 and RBCK1 occurs through binding of the ubiquitin-associated (UBA) domain of RNF31 with the ubiquitin-like (UBL) domain of RBCK1 (6). The specific binding between these two domains is critical for the formation of the linear ubiquitin chain assembly complex (LUBAC). This complex has the unique ability to catalyze the formation of linear peptide bonds between the amino-terminal methionine of one ubiquitin molecule and the carboxyl-terminal glycine of the adjacent ubiquitin (7). Recently, SHARPIN was identified as a third component of active LUBAC (8–10). Linear ubiquitin chains are the only known products of LUBAC and have an important role as regulators of cellular signaling pathways that control innate immunity and inflammation through NF-κB activation (5–10). Although their role in pathologies and diseases is still poorly understood, LUBAC is undoubtedly involved in inflammatory diseases and cancer. Therefore, the development of new drugs targeted to LUBAC components may prove useful for therapeutic purposes.

In their article, Yang and colleagues (3) established a clear causative link between LUBAC and the pathogenesis of ABC DLBCL. In these cells, constitutive activation of the NF-κB pathway plays an important role in their cancerous behavior. In fact, the malignancy of ABC DLBCL is a consequence of the persistent activation of the B-cell receptor (BCR) and MYD88 signaling, with 20% of oncogenic cells presenting mutations in the immunoreceptor tyrosine-based activation motifs (ITAM) of the BCR subunits CD79B and CD79A and 10% presenting mutations in CARD11. CARD11 forms a multiprotein complex with MALT1 and BCL10, determining, through the activation of IkB kinase, the activation of the classical NF-κB signaling. Moreover, a somatic gain-of-function mutation of MYD88 is also responsible for the activation of the NF-κB pathway in a large percentage of ABC DLBCL lymphoma cells (11).

Yang and colleagues (3) performed a genetic screen on ABC DLBCL patient biopsies, focusing on genes coding for the LUBAC complex proteins, and identified two recurrent missense mutations, which were previously annotated as rare SNPs. These genetic variations result in a change of glutamine 584 to histidine (Q584H) and glutamine 622 to leucine (Q622L) in the UBA domain of RNF31. The UBA is a highly conserved region responsible for the interaction of RNF31 with RBCK1 and consequent LUBAC activation (6). The two SNPs resulted in a stronger interaction between RNF31 and RBCK1, with a consequent increase in E3 ligase activity of the complex. The exact mechanism by which the two SNPs affect the complex formation is still unclear, but the analysis of their position in the crystal structure suggests a significant modification in the structure of RNF31. Both mutations are located at peculiar sites: Q622L is set in an unusual bent α-helical region and...
Q584L in the α6 helix. In both cases, the alteration generates a modification in the protein conformation of RNF31, which could be responsible for a stronger electrostatic interaction with RBCK1. The natural consequence of this stronger link is increased linear ubiquitination by LUBAC, which determines the persistent activation of NF-κB signaling.

The authors elegantly demonstrated the direct involvement of LUBAC in the regulation of NF-κB signaling in ABC DLBCL cells. RNF31 and SHARPIN keep the NF-κB pathway active by maintaining constitutively linear ubiquitination of the IKKγ/NEMO subunit. The RNF31 SNPs affect NF-κB activity on multiple levels. In ABC DLBCL cells, mutant RNF31 increased the expression of the two well-known NF-κB target genes NFKBIA and IRF4, and at the same time positively stimulated inhibitor of IKK binding activity. Moreover, the abnormal LUBAC activity contributes to the increased linear ubiquitination of NEMO, stimulating recruitment of A20 to the CARMA1–BCL10–MALT1 (CBM) complex, leading to its consequent cleavage.

Although the authors did not provide a clear molecular picture to explain the recruitment of A20 to the CBM complex, they did propose two interesting theories. One possibility is that the recruitment of A20 to the CBM complex is determined by ubiquitination of the receptor complex proteins by LUBAC. The consequence of increased avidity of the CBM complex for A20 results in its cleavage mediated by MALT1 and CARD11. An alternative explanation is that LUBAC-mediated ubiquitination of the CBM complex increases the proteolytic activity of MALT1 by a yet-unidentified mechanism.

These new findings implicate LUBAC in the regulation of oncogenic signaling and open new avenues to therapeutically targeting the cancerous pathology. In their article, the authors adopted an interesting strategy for modulation of LUBAC activity. Using “hydrocarbon stapling,” which stabilizes peptides forming α-helical structures, they were able to generate a peptide, RNF31 N-Q622L, with increased α-helical character. This “stapled” peptide interacted more with RBCK1 and outcompeted the wild-type RNF31 peptide in preventing...
endogenous LUBAC formation in ABC DLBCL cells. Treatment with the peptide resulted in reduced activity of the NF-κB pathway, which is the main survival pathway for the lymphoma cells, and increased the effect of chemotherapeutic drugs (Fig. 1). Additional analysis using in vivo ABC DLBCL cell grafting would be extremely useful to evaluate the quality and the safety of this approach. Moreover, as the authors discussed, it is important to emphasize that LUBAC does not affect all signaling events in the NF-κB pathway. Drugs inhibiting LUBAC could have different effects, other than IKKβ, so their applications could be even more interesting and diverse.

The overall data provided in the article by Yang and colleagues (3) lead to new and exciting questions about the role of LUBAC in the regulation of the NF-κB pathway through signaling by the CBM complex. Moreover, the development of stapled peptides opens interesting avenues for the possible use of small-molecule inhibitors to counteract the oncogenic role of E3 ligases in various malignancies. Of note, this is the first time that a therapeutic treatment targeting the LUBAC complex has been proposed and could have additional important clinical applications. The regulatory role of LUBAC on BCR and Toll-like receptor signaling also makes this complex an attractive pharmacologic target for inflammatory diseases. Last but not least, this study shows that the continued investigation of SNPs may lead to valuable insights in the pathogenesis of cancer and other genetically modulated diseases, and has the potential to drive the field forward.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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