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LYMPHOID NEOPLASIA

Comment on Sievers et al, page 1293

CRISPRing the CRL4^{CRBN} RING in multiple myeloma

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In this issue of *Blood*, Sievers et al describe key proteins required for the anti-myeloma properties of lenalidomide. By performing CRISPR-Cas9 functional genetic screening, they identify 2 E2 ubiquitin-conjugating enzymes (UBE2D3 and UBE2G1) and the COP9 signalosome as essential for lenalidomide-dependent CRL4^{CRBN} function in myeloma due to their regulation of CRL4^{CRBN} activity and ubiquitination of target substrates.¹

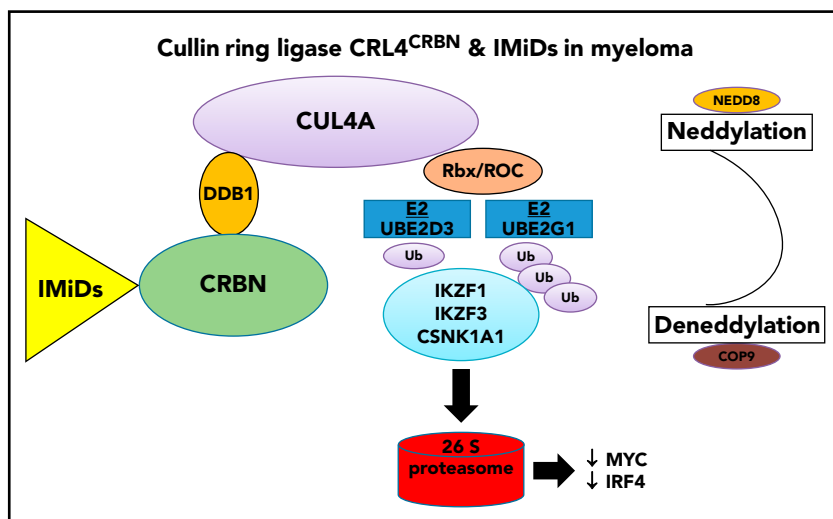
The story of immunomodulatory drugs (IMiDs), such as thalidomide, lenalidomide, and pomalidomide, in multiple myeloma (MM) is quite surreal. In the late 1950s, thalidomide was used to treat

morning sickness but was discontinued in the early 1960s after it was found to cause birth defects. Despite its tragic past, thalidomide has reentered clinical practice due to its immunomodulatory and

antiangiogenic properties and found to be effective in the treatment of MM in 2006 when was approved by the Food and Drug Administration for the treatment of patients with relapsed MM. Since then, its derivatives lenalidomide and pomalidomide have proven their activity and are now widely used in newly diagnosed and relapsed refractory MM. In 2010, Ito et al² reported that cereblon (CRBN), a substrate receptor of the CRL4 E3 ubiquitin ligase, was a direct target of IMiDs and was required for the teratogenic activities of thalidomide. We also now recognize that in MM cells IMiDs bind to CRBN and neomorph the substrates recruitment to the Cul4A Cullin ring E3 ligase, inducing Ikaros (IKZF1), Aiolos (IKZF3), zinc finger protein 91 (ZFP91), and casein kinase 1 α (CSNK1A1) ubiquitylation and proteasomal degradation. These events lead to specific and sequential transcriptional repression of MYC and IRF4, 2 essential factors for myeloma cells' survival.^{3,4} However, it is not clear how myeloma cells acquire resistance to IMiDs, "beyond CRBN," and whether other neo-substrates essential for MM survival are targeted by IMiDs for degradation.

In the last few years, clustered regularly interspaced short palindromic repeats-CRISPR associated nuclease 9 (CRISPR-CAS9) systems have emerged as versatile, reliable, and convenient genome-editing tools and have opened a new era in molecular biology.⁵ Herein, by performing a genome-scale CRISPR-CAS9 screen in lenalidomide-sensitive MM cell lines, Sievers et al identified genes that, when inactivated, diminish the effects of lenalidomide and investigated their roles in the modulation of the CRL4^{CRBN} ubiquitin ligase.

The ubiquitin proteasome system is an important posttranslational regulatory process for proteins that alters their stability, localization, or interaction properties.⁶ It is mediated by the successive enzymatic reactions involving activating enzymes, conjugating enzymes (E2), and ligase enzymes (E3), resulting in an isopeptide link between the C-terminus glycine residue of ubiquitin and a specific lysine on a target protein.⁷ In this process, the E3 ligase determines the substrate specificity and stability. Among the diverse E3 enzymes, the cullin-RING ubiquitin ligases are the largest E3 ligase family in eukaryotes and can be regulated by a process termed neddylation that involves cullin-associated protein Nedd8⁸



Schematic representation of the CRL4^{CRBN} complex in myeloma cells. IMiDs bind to CRBN, a substrate receptor protein for the CRL4 E3 ubiquitin ligase complex, and cause proteasomal degradation of IKZF1, IKZF3, and CSNK1A1 consecutively downregulating IRF4 and Myc and resulting in MM-associated cytotoxicity. The blue boxes indicate the 2 E2 ubiquitin-conjugating enzymes, UBE2D3 and UBE2G1, reported by Sievers et al as essential for lenalidomide-dependent CRL4^{CRBN} function in MM.

and neddylation, which removes the Nedd8 moiety and requires the isopeptidase activity of the COP9 signalosome.⁹ Among the 6 human cullins family, the cullin4 (CUL4) subfamily comprises 2 members, CUL4A and CUL4B, which share 83% sequence identity and functional redundancy. It consists of a RING finger domain protein, CUL4 scaffold protein, and DDB1-CUL4 associate substrate receptors. Recent studies have highlighted the role of CUL4A complexes in regulating substrates involved in the cell cycle, signaling, tumor suppression, DNA damage response, and chromatin remodeling and suggested CRL4A as a promising novel target for cancer therapy.¹⁰

CRBN is a substrate receptor protein for the CRL4A E3 ubiquitin ligase complex, and drugs like IMiDs have been reported to be able to inhibit or alter the substrate specificity of the E3 ligase activity of CRL4A^{CRBN}. Therefore, the identification of the molecular components that regulate IMiDs-dependent activity of CRL4^{CRBN} will allow a better understanding of the parameters controlling their therapeutic efficacy and help to identify the mechanisms underlying their resistance. As such, through an elegant genome-wide CRISPR-CAS9 screen, the authors identified regulators of cullin-RING ligase neddylation as well as the elusive CRL4A^{CRBN} E2 conjugating enzymes. By using functional genetics and in vitro assays, they demonstrated that UBE2D3 primes CRL4^{CRBN} target substrates via monoubiquitination, after which UBE2G1 polyubiquitinates them via K48-linked ubiquitin chains. Furthermore, loss of UBE2M or members of the COP9 signalosome resulted in altered neddylation of CUL4A and impaired lenalidomide-dependent CRL4^{CRBN} activity (see figure).

Overall, the results presented here are novel and pivotal to expand our understanding of IMiDs' mechanisms of action and likely to elucidate mediators of acquired resistance. Additional questions remain, including whether other CRL4-containing E3 ligases utilize UBE2G1/UBE2D3 conjugating enzymes and what structural motifs on CRL4^{CRBN} or its substrates dictate E2 usage and specificity. Nevertheless, Sievers et al, by CRISPRing the CRL4^{CRBN} ring, established key proteins required for lenalidomide-dependent CRL4^{CRBN} function in MM and provided us with a deeper understanding of this ubiquitin ligase function and regulation. The next step will be to therapeutically

exploit these newly identified targets to augment or restore the activities of IMiDs in MM.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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PHAGOCYTES, GRANULOCYTES, AND MYELOPOIESIS

Comment on Bellanné-Chantelot et al, page 1318

SRP54 and a need for a new neutropenia nosology

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In this issue of *Blood*, Bellanné-Chantelot et al noted that mutations in signal recognition particle 54 (SRP54) cause an inherited neutropenia in 23 individuals with features of both severe congenital neutropenia (SCN) and Shwachman-Diamond syndrome (SDS).¹

In fact, after *ELANE*, mutations in *SRP54* are the second most common cause of inherited neutropenia in the French Congenital Neutropenia Registry. Together with the 3 patients reported by Carapito et al,² the work of Bellanné-Chantelot et al adds to our understanding of genetic pathways and phenotype correlations of inherited neutropenias, but it raises the question of how to classify these and other genetic cytopenias: molecular pathway-based classification or phenotype-based classification?

The inherited bone marrow failure syndromes constitute a heterogeneous group of blood disorders that are phenotypically distinct.³ They are true experiments of nature, enlightening our understanding

of normal and pathologic hematopoiesis and often non-hematopoietic tissue development. Their importance goes further with their acting as leukemia and/or cancer predisposition syndromes. Molecular cloning has reinforced phenotype distinctions by revealing that mutations in pathways lead to the separate clinical entities. Fanconi anemia is caused by at least 20 different genes involved in DNA damage response, Diamond-Blackfan anemia by at least 15 genes involved in ribosomal structure, and dyskeratosis congenita by at least 10 genes that promote telomere maintenance and stability.

The inherited neutropenias include SCN, cyclic neutropenia (CyN), SDS, and a motley