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Novel insights into the mechanism of action of lenalidomide

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Abbreviations: CRBN, cereblon; FDA, Food and Drug Administration; IFR4, interferon-regulatory factor 4; IKZF, IKAROS family zinc finger; IMiD, immunomodulatory drug; MM, multiple myeloma; shRNA, short-hairpin RNA

Lenalidomide (Revlimid®) is a synthetic derivative of thalidomide (Thalomid®) currently licensed by the US Food and Drug Administration (FDA) and other international regulatory agencies for the treatment of multiple myeloma (MM) (in combination with dexamethasone)[1-3] and low or intermediate-1 risk myelodysplastic syndromes bearing 5q cytogenetic abnormalities (as a standalone agent).[4-6] Lenalidomide was originally developed to improve the safety profile of thalidomide, which eventually turned out to be responsible for an estimated number of 10000–20000 phocomelic babies worldwide in the 1950–60s.[7] As compared with thalidomide, lenalidomide is associated with limited neurotoxic activity, but is not completely devoid of teratogenic effects.[8-10] For this reason, both thalidomide (which is also approved for use in MM patients, together with dexamethasone)[11] and lenalidomide are commercialized under tightly controlled distribution programs (as per explicit request of the US FDA).[8] Recently, a novel thalidomide derivative, pomalidomide (Pomalyst®), has been approved for the treatment of specific subsets of MM patients, including individuals who progressed on or shortly after thalidomide therapy.[12,13] At odds with thalidomide and lenalidomide, pomalidomide exerts very limited (if any) teratogenic activity.[14]

Throughout the 2000s, thalidomide and lenalidomide have been the subjects of an intense wave of investigation, revealing multiple biological effects that could account for their antineoplastic activity.[15-17] In particular, lenalidomide has been shown to (1) limit the proliferation of cancer cells and promote their death; (2) interrupt the trophic support provided to malignant cells by the tumor stroma; and (3) operate as a pleiotropic immunomodulator.[18,19] Owing to their ability to boost both the innate and adaptive arm of the immune response, thalidomide, lenalidomide, and pomalidomide are collectively referred to as immunomodulatory drugs (IMiDs).[20,21]

In spite of the acute interest generated by IMiDs throughout the past decade, the actual molecular target of these drugs has been discovered only in 2010, when thalidomide was shown to physically interact with (and hence inhibit) the E3 ubiquitin ligase cereblon (CRBN).[22] Zebrafish (*Danio rerio*) embryos exposed to thalidomide developed limb abnormalities that mimicked human phocomelia, unless they expressed a variant of CRBN that does not bind IMiDs ($CRBN^{YWAA}$).[22] Importantly, developmental limb defects also manifested in CRBN-deficient zebrafish, indicating that the teratogenic effects of thalidomide stem from CRBN inhibition.[22,23] Apparently in contrast with this notion, lenalidomide and pomalidomide were subsequently suggested to stabilize CRBN,[24] thus decreasing the half-life of several proteins that are normally degraded by the ubiquitin-proteasome system, including interferon-regulatory factor 4 (IRF4).[24,25] Of note, the stable depletion of CRBN endowed cultured cancer cells with a pronounced resistance to IMiDs. Moreover, the neoplastic compartment of MM patients who failed to respond to lenalidomide-based therapy was found to express low CRBN levels after treatment.[25] These findings indicated that the antineoplastic activity of IMiDs actually requires CRBN, a notion that has recently been confirmed and expanded by 2 independent studies.[26,27]

By means of 2 distinct experimental approaches, i.e., stable isotope labeling of amino acids in cell culture (SILAC)-based quantitative proteomics and a firefly luciferase-based approach to monitor protein stability, the groups headed by Benjamin L Ebert and William G Kaelin Jr demonstrated that the CRBN-dependent antineoplastic activity of lenalidomide originates from the degradation of 2 lymphoid transcription factors, namely, IKAROS family zinc finger 1 (IKZF1, also known as Ikaros) and IKZF3 (also known as Aiolos).[26,27] In particular, lenalidomide (as well as thalidomide and pomalidomide) was found to increase the binding of CRBN to IKZF1 and IKZF3 (but not other Ikaros family members), thus promoting their proteasomal degradation.[26,27] In line with previous findings,[24,25] such an effect could be abrogated by the depletion of CRBN with short-hairpin RNAs (shRNAs), rendering MM cells resistant to the cytotoxic activity of lenalidomide. Similar

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results were obtained with *CRBN*$^{-/-}$ cells as well as with *CRBN*$^{-/-}$ cells expressing CRBN^{YWAA}.[26,27] Conversely, the complementation of *CRBN*$^{-/-}$ cells with wild-type CRBN restored the sensitivity of IKZF1 and IKZF3 to lenalidomide-triggered proteasomal degradation.[27]

In addition, the groups lead by Benjamin L Ebert and William G Kaelin Jr succeeded in mapping the residue of Ikaros family members that is responsible for their differential sensitivity to lenalidomide-triggered degradation. Thus, at odds with their wild-type counterparts, IKZF3^{Q147H} and IKZF4^{H188Q} were shown to be insensitive and sensitive, respectively, to lenalidomide.[26,27] Importantly, the shRNA-mediated depletion of IKZF1 or IKZF3 was sufficient to stimulate the demise of lenalidomide-sensitive cells, an effect that was mimicked by a dominant negative variant of IKZF3 lacking the DNA-binding domain. Conversely, the overexpression of wild-type IKZF3 (and more so that of degradation-resistant IKZF mutants, i.e., IKZF1^{Q146H} and IKZF3^{Q147H}) protected MM cells against lenalidomide cytotoxicity.[26,27]

The degradation of IKZF1 and IKZF3 in IMiD-sensitive cells exposed to lenalidomide was often followed by a decline in the levels of IRF4,[26,27] a transcription factor that had previously been involved in the mechanism of action of this drug.[25,28,29] However, some cell lines succumbed to lenalidomide in the absence of appreciable IRF4 downregulation, suggesting that the direct antineoplastic effects of IMiDs involve at least another molecular target of Ikaros family members. Finally, T cells exposed to lenalidomide secreted high amounts of interleukin-2, a potent immunostimulatory cytokine,[30,31] as a correlate of IKZF1/IKZF3 degradation,[26] reinstating the notion that the biological activity of IMiDs is broad and involves a non-negligible immunological component.[32,33]

In summary, the research units headed by Benjamin L Ebert and William G Kaelin Jr have provided novel insights into the mechanism of action of lenalidomide and other IMiDs, possibly identifying a therapeutic window to definitively discriminate between their teratogenic and therapeutic effects. What remains to be precisely elucidated is to which extent the degradation of IKZF1 and IKZF3 as triggered by lenalidomide mediates antineoplastic effects via cancer cell-intrinsic vs. immunological mechanisms. Moreover, it will be interesting to understand whether polymorphisms in *IKZF1*, *IKZF3,* and/or genes encoding their transcriptional targets influence the propensity of individual MM patients to respond to lenalidomide. Well-designed preclinical and clinical studies are required for addressing these hitherto unresolved issues.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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