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HAEMATOLOGICAL CANCER

Ikaros—not a myth for myeloma

Lenalidomide is a powerful drug for the treatment of multiple myeloma and other B-cell lymphomas. Two independent groups, respectively, led by Benjamin Ebert and William Kaelin, recently reported that degradation of the ikaros proteins, IKZF1 and IKZF3, is the main event underlying lenalidomide antineoplastic function, finally shedding some light on the mechanisms of action of this drug.

The remarkable clinical activity of lenalidomide and its analogues thalidomide and pomalidomide (collectively immunomodulatory drugs or IMiDs) is eclipsed by their teratogenic effect, which limits their use. Recent studies have demonstrated that IMiDs bind to and inactivate the cereblon E3 ubiquitin ligase and that loss of cereblon could explain the teratogenicity of the drugs. "In myeloma cells, high levels of cereblon are associated with responsiveness, while a low level of cereblon renders cells resistant to IMiD treatment, indicating that the therapeutic activity of thalidomide-like drugs is actually due to a new activity of cereblon when bound to these agents," explains Kaelin.

The E3 ubiquitin ligase complex includes cereblon, damaged DNA binding protein 1, Cullin-4A, and regulator of cullins 1. The E3 enzymes are responsible for the substrate specificity of the ubiquitination reaction but, until now, no substrate had been identified for this complex. Kaelin *et al*. transfected 293FT cells with a plasmid library encoding 15,483 open reading frames (ORFs) fused to luciferase and looked for proteins whose stability was influenced by lenalidomide treatment. As expected, most ORFs were unaffected by lenalidomide treatment, but 107 ORFs were subjected to a secondary screen that ultimately led to the identification of IKZF1 and IKZF3 as proteins downregulated upon drug administration.

Ebert and colleagues reached similar conclusions using a different approach. To understand how lenalidomide alters cereblon ubiquitin ligase function the researchers "used a series of quantitative global proteomic approaches, examining the effects of the drug on ubiquitinated proteins, the level of such proteins in the cell and the proteins bound to the cereblon substrate adapter." Ebert continues, "all these approaches pointed to the same two proteins, IKZF1 and IKZF3, as lenalidomide-regulated targets of the cereblon ubiquitin ligase."

Both groups suggest that lenalidomide directly binds cereblon and showed that the binding of IKZF1 and IKZF3 (but not other members of the ikaros protein family) to cereblon was enhanced in the presence of lenalidomide, promoting an increased ubiquitination of its targets and subsequent degradation. Importantly, both studies demonstrated that the lenalidomide-dependent degradation of IKZF1 and IKZF3 relies on the presence of cereblon. In fact, loss of *cereblon* or the expression of a mutated protein that does not bind lenalidomide fails to promote the drug-dependent degradation of the ikaros targets. As Kaelin emphasizes, "the teratogenic effects of IMiDs are due to inactivation, or loss, of the cereblon ubiquitin ligase … the therapeutic activity of IMiDs is actually due to a cereblon gain of function. Infact, cereblon bound to these drugs acquires the ability to destroy IKFZ1 and IKFZ3, two B-cell transcription factors that have a critical role in myeloma." Crucially, IKFZ1 and IKFZ3 would not normally be targeted for degradation.

These reports demonstrate that the teratogenic and antineoplastic activities of IMiDs can be dissociated. Additionally, understanding both molecular mechanisms underlying these effects will help in the design of drugs that avoid the teratogenicity and enhance the efficacy. Finally, these studies reveal a new mechanism of action that is perhaps shared by other drugs and might, according to Kaelin, provide a new paradigm for drugging undruggable proteins.

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Original articles Kronke, J. *et al*. Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells. *Science* doi:10.1126/science.1244851 | Lu, G. *et al*. The myeloma drug lenalidomide promotes the cereblon-dependent destruction of ikaros proteins. *Science* doi:10.1126/science.1244917