Chemical control over protein abundance is a desirable therapeutic strategy that has the potential to disrupt molecular processes and activities organized not via enzymatic turnover but at the level of intermolecular interactions. In contrast to conventional small-molecule inhibition, protein degradation offers a means to disrupt scaffolding functions underpinning intermolecular crosstalk. To expand on the mechanism of IMiD-based chemical reprogramming of CRL4<sub>CRBN</sub>, a conjugation strategy using bifunctional ligands was developed<sup>6,7</sup>. In brief, an IMiD-like moiety is conjugated to a targeting ligand known to bind a protein of interest (POI). Resulting bifunctional compounds induce molecular proximity of CRL4<sub>CRBN</sub> and the POI, leading to ubiquitination and degradation of the POI. A comparable strategy has been devised to recruit CRL2<sub>β</sub> on the basis of an optimized small-molecule binding to VHL<sub>α</sub>-<sub>10</sub>. The modularity of these approaches allows them to be extended by altering the targeting ligand to potentially degrade a wide target space. However, the high molecular weight of bifunctional molecules and the incompletely understood steric requirements for successful POI recruitment and ubiquitination bear potential future limitations. Further extension of chemical control over additional E3 ligases is therefore vital for the field and for further clinical translation.

Two recent studies report orthogonal approaches leading to the identification of the mechanism of action of indisulam and other sulfonamides as modulators of the E3 ligase complex DCAF15 (refs. 4,5). Both studies set out to elucidate the enigmatic antiproliferative effect of indisulam and other sulfonamides. Whereas Uehara <i>et al.</i> employed a target-identification campaign based on expression proteomics, Han <i>et al.</i> established drug-tolerant clones and identified causative genomic aberrations using exome sequencing as a means for target identification. Both strategies recognized that sulfonamides induce proteasomal degradation of the splicing factor RBM39 (also known as CAPERα). Immunoprecipitation of RBM39 coupled with mass spectrometry revealed that sulfonamide treatment leads to the recruitment of RBM39 to the CRL4<sub>DCAF15</sub> complex via ligand-mediated, direct interaction between RBM39 and DCAF15 (Fig. 1b). RBM39 recruitment causes its CRL4<sub>DCAF15</sub>-dependent ubiquitination and degradation. Further experimental evidence validated that loss of RBM39 is causative for the compound’s antiproliferative effect. Han <i>et al.</i> observed that depletion of RBM39 led to pronounced splicing defects, and that hematopoietic and lymphoid cell lines are disproportionally sensitive to indisulam treatment. Correlating indisulam efficacy with overall gene expression led to the identification of DCAF15 expression as a biomarker that is predictive for drug response. These findings open up a multitude of follow-up questions. What are the selectivity and extensibility of this process? Is RBM39 the only target, or could CRL4<sub>DCAF15</sub> be modulated further to degrade other...
proteins? Future studies will shed light on the translational relevance of degrading RBM39 in hematopoietic malignancies, focusing especially on cases with pre-existing mutations in the splicing machinery. Han et al. report that splicing defects caused by acute RBM39 degradation are focal and affect only a subset of genes. This potentially enables a more selective targeting of a dependency of hematopoietic cancers on splicing. Understanding the mechanistic basis for this observed selectivity is very intriguing, and it warrants further investigation and renders indisulam an attractive chemical probe to study splicing.

Compared to the relatively narrow SAR of IMiDs, the reported sulfonamides are chemically more diverse, and structural determinations of sulfonamide-induced target recognition will be valuable in further dissection of the SAR inherent to CRL4<sup>DCAF15</sup> modulation. A biotinylated sulfonamide derivative with retained target engagement was reported by Uehara et al., suggesting that converting sulfonamides into bifunctional molecules is a valid option for modulating CRL4<sup>DCAF15</sup> in a rational manner on the basis of the choice of the respective targeting ligand. Further insights into tissue distribution and subcellular localization of DCAF15 will be required to evaluate the feasibility of achieving tissue- or compartment-specific degradation. Finally, further increasing the arsenal of ‘reprogrammable’ E3 ligases will be vital for cancer therapy, as loss of the respective E3 substrate receptor is a predictable resistance mechanism that can be overcome by altering which ligase complex is recruited.

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