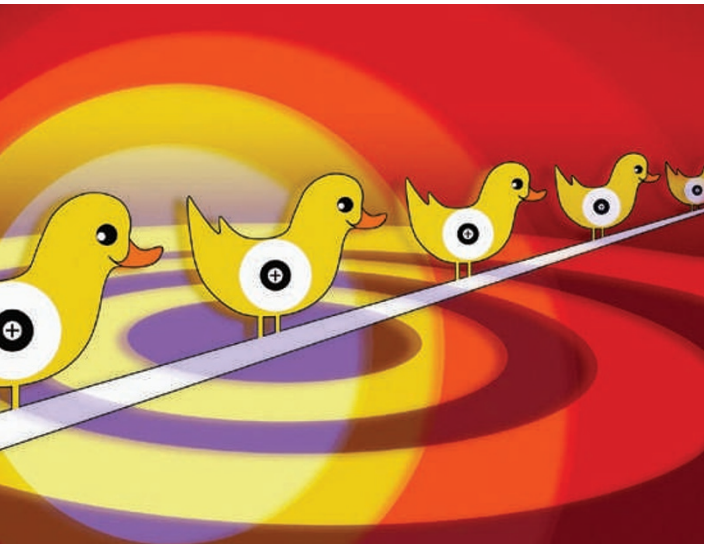
ANTICANCER DRUGS

Selectively targeting turnover

Targeting the ubiquitin–proteasome system (UPS) has proved clinically useful in cancer, with the proteasome inhibitor bortezomib approved for the treatment of multiple myeloma and relapsed mantle cell lymphoma. Writing in *Nature*, Soucy *et al.* describe a promising approach to targeting protein turnover more specifically: inhibition of NAE (NEDD8-activating enzyme).

NAE catalyses the first step in a conjugation pathway that is necessary for the activity of a subtype of ubiquitin E3 ligases, the cullin–RING ligases (CRLs): the ubiquitylating activity of CRLs depends on neddylation of the cullin subunit. CRLs in turn regulate the degradation of a subset of proteins by the proteasome. The authors identified the molecule MLN4924, an AMP analogue, as an inhibitor of NAE and showed its selectivity for NAE over the related enzymes ubiquitin-activating enzyme, SUMO-activating enzyme, UBA6 and autophagy-related protein 7. By comparing the effect of this agent with that of bortezomib in HCT-116 cells, the authors were able to show that ~20% of UPS-mediated protein turnover is attributable to NEDD8-dependent CRL activity. MLN4924 treatment of HCT-116 cells resulted in a rapid dose-dependent decrease of complexes of NEDD8 with both UBC12 (the E2 enzyme to which NAE transfers NEDD8) and cullin, and an increase in the concentrations of several CRL substrates, including NRF2 (also known as NEF2L2) and chromatin-licensing and DNA replication factor 1 (CDT1), but not non-CRL substrates.



The proteins ubiquitylated by CRLs are varied, so the possible cellular outcomes of preventing CRL activity are manifold. The authors used MLN4924 to reduce NEDD8–cullin levels and observed an accumulation of cells in S phase and a large proportion of cells with ≥4*N* DNA content. This phenotype, which was reproduced in various human tumour cell lines, is similar to that of cells undergoing rereplication — initiation of multiple rounds of DNA replication without cell cycle progression — and the observed accumulation of CDT1, a DNA replication licensing protein, suggested that the effect might be mediated by this molecule. Forced expression of CDT1 or RNA interference-mediated ablation of its inhibitor has previously been shown to produce the same phenotype. After 48 hours there was an increase in the number of cells with <2*N* DNA content, indicating that cells were undergoing apoptosis. Further investigation is necessary to determine the mechanism by which MLN4924 treatment causes cell death, although one possibility was suggested by the dose-dependent accumulation of DNA damage signalling proteins, including phosphorylated CHEK1.

So, do these results translate into a useful effect *in vivo*? Analysis of tumours from mice treated with MLN4924 showed that the pathway responses were comparable to those observed in cultured cells, with a single dose causing a decrease in NEDD8–cullin levels, increased steady state levels of NRF2 and CDT1, and an increase in phosphorylated CHEK1. Furthermore, the growth of HCT-116 xenografts in mice was significantly inhibited by the administration of various dosing schedules of MLN4924, and H522 lung tumour xenografts displayed almost complete regression. Moreover, the treatment was well tolerated.

Overall these results suggest that inhibition of NAE might be a viable method of targeting the UPS in cancer, with useful differences in specificity and safety profiles compared with existing treatments.

Isobel Barry
Nature Reviews Cancer

ORIGINAL RESEARCH PAPER Soucy, T. A. *et al.* An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. *Nature* **458**, 732–736 (2009)