ANTICANCER DRUGS

Chasing elusive targets

Major drivers of human cancer include members of the families of MYC proteins and RAS-like GTPases, which are commonly over-expressed and amplified in tumours. However, despite their prominence in cancer, agents capable of therapeutically targeting these proteins have so far proved elusive. Now, two papers each describe a novel strategy to identify such agents — targeting specific MYC and RAS proteins, respectively — and show that they prevent tumour growth in mouse models.

In the first paper, Gustafson *et al.* focus on targeting the MYC family member MYCN, which is frequently amplified in neuroblastoma and associated with tumour progression and poor outcome. MYCN is stabilized by Aurora A in a kinase-independent manner involving a protein–protein interaction. The authors therefore set out to disrupt MYCN–Aurora A binding, with the aim of promoting MYCN destabilization and degradation.

Hypothesizing that MYCN stabilization requires a distinct conformation of Aurora A, they synthesized and screened a set of compounds that were predicted to induce a large structural shift in Aurora A. CD532 — which features an aminopyrazole–pyrimidine ATP-mimetic backbone — was identified as the most active compound.

Analysis of the crystal structure of the catalytic domain of Aurora A bound to CD532 revealed that CD532 binds to Aurora A at the hinge region via a pyrazole moiety. This interaction stabilized an inactive DFG-in conformation of Aurora A that was associated with a 6.2 Å shift in the position of the amino-terminal domain relative to the carboxy-terminal domain, a disengaged state of the regulatory HRD motif and a 180° flip in the activation loop.

In MYCN-amplified neuroblastoma cells, CD532 potentially inhibited aurora A activity, enabling proteasomal degradation of MYCN, and was cytotoxic. And in mice with MYCN-amplified neuroblastoma xenografts, 2 day treatment with CD532 decreased levels of MYCN protein. Furthermore, in mice with MYCN-expressing medulloblastoma allografts, twice weekly CD532 treatment for 3 weeks reduced MYCN levels, decreased tumour burden and extended survival, without signs of toxicity.

Meanwhile, Yan *et al.* set out to target the RAS-like GTPases, RALA and RALB, which promote proliferation, survival and metastasis of multiple human cancers. The authors hypothesized that molecules that selectively bind to RAL–GDP might restrict RAL to an inactive state in the cell.

Comparison of the available three-dimensional structures of RALA revealed a potentially targetable region formed by the switch-II region, the $\alpha 2$ helix and one face of the $\alpha 3$ helix, which was adjacent to, but distinct from, the guanine nucleotide binding pocket. Using a structure-based virtual screening approach, they assessed 500,000 small molecules for their ability to bind to this site and identified 88 hit compounds.

In human bladder cancer cells expressing RALA, three of these compounds — RBC6, RBC8 and RBC10 — reduced binding of RALA to its effector protein RALBP1, inhibited activation of RALA and prevented RAL-mediated cell spreading of mouse embryonic fibroblasts.

Synthesis of a series of RBC8 derivatives produced BQU57, which exhibited superior *in vitro* RAL binding and drug-like properties. Allosteric binding of BQU57 to RALB–GDP was confirmed by isothermal titration calorimetry, surface plasmon resonance and nuclear magnetic resonance spectroscopy. Both RBC8 and BQU57 blocked RALA and RALB activation in human lung cancer cell lines, inhibiting anchorage-independent growth.

In mice with human lung cancer xenografts, 21 day treatment of RBC8 or BQU57 specifically blocked RALA and RALB, inhibiting tumour growth to a similar extent to dual knockdown of RALA and RALB.

In summary, these studies have identified novel strategies for targeting oncoproteins that have been previously deemed undruggable, which may have implications for the future treatment of numerous human cancers.

Sarah Crunkhorn

ORIGINAL RESEARCH PAPERS Gustafson, W. C. *et al.* Drugging MYCN through an allosteric transition in Aurora kinase A. *Cancer Cell* **26**, 414–427 (2014) | Yan, C. *et al.* Discovery and characterization of small molecules that target the GTPase RAL. *Nature* <http://dx.doi.org/10.1038/Nature13713> (2014)