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SIGNALLING

Putting the brakes on KRAS-G12C nucleotide cycling

The KRAS-G12C mutation occurs in ~20% of lung cancers; however, targeting this small GTPase has been challenging. Two new studies have characterized a potent mutant-selective inhibitor and provide novel insights into the function of KRAS-G12C.

Patricelli, Janes *et al.* set out to design a drug to target a recently discovered binding pocket — the Switch II pocket — of KRAS-G12C. They developed a mass spectrometry-based assay to directly quantify KRAS-G12C activity and identified a novel potent inhibitor — ARS-853 — for further study. They confirmed selective covalent binding of ARS-853 to the Switch II pocket using high-resolution crystal structure analysis.

Patricelli, Janes *et al.* and Lito *et al.* both showed that ARS-853 treatment of multiple KRAS-G12C-expressing lung cancer cell lines (but not cells lacking KRAS-G12C) markedly and dose-dependently reduced the levels of GTP-bound KRAS and the extent of phosphorylation and/or interaction with downstream effectors, including CRAF, ERK and AKT. Furthermore, ARS-853 induced apoptosis (both studies), decreased growth in both 2D and 3D assays (Patricelli, Janes *et al.*) and reduced proliferation (both studies); the effects on growth and proliferation could be rescued by ectopic expression of G12V-mutant KRAS (Patricelli, Janes *et al.*), further confirming the selectivity of ARS-853 for KRAS-G12C.

Both groups went on to investigate the mechanism of action of ARS-853, and data from thermal stability assays (Lito *et al.*) and structural and biochemical studies (Patricelli, Janes *et al.*) indicated that ARS-853 preferentially binds to GDP-bound KRAS-G12C. Notably, these data contradict the widely held view that activating mutations in KRAS 'lock' the enzyme in the active GTP-bound form that drives constitutive oncogenic signalling. Both groups showed that KRAS-G12C is able to cycle between GDP- and GTP-bound states and that ARS-853 inhibits this cycling by locking the enzyme in the inactive GDP-bound form. In support of this, Lito *et al.* showed, using mass spectrometry and cell-based assays, that the GTPase activity of KRAS-G12C (and thus formation of the inactive GDP-bound enzyme) is required for ARS-853-mediated inhibition.

Lito *et al.* hypothesized that the extent of inhibition could be influenced by the activity of guanine nucleotide exchange factors (GEFs; such as SOS), which regulate cycling by promoting GTP binding. Indeed, ARS-853 reduced interaction between KRAS-G12C and SOS, as well as SOS-induced nucleotide exchange, suggesting that ARS-853 lowers the affinity of KRAS-G12C for SOS. Furthermore, using distinct assays, both studies demonstrated that ARS-853-mediated inhibition of KRAS-G12C was reduced when levels of nucleotide exchange were increased (for example, by exposing cells to epidermal growth factor (EGF), which activates SOS), and was enhanced when levels of exchange were reduced (for example, by exposing cells to an EGF receptor inhibitor). These data suggested that ARS-853 competes with GEFs to bind KRAS-G12C and indicated that rather than being constitutively active, KRAS-G12C is responsive to upstream signalling pathways known to regulate wild-type KRAS. Notably, concomitant inhibition of EGF signalling enhanced the effects of ARS-853 on the proliferation (Lito *et al.*) and apoptosis (both studies) of KRAS-G12C-expressing lung cancer cell lines.

In summary, both studies identify ARS-853 as a potent and selective inhibitor of KRAS-G12C, but further work is needed to determine whether ARS-853 can inhibit the *in vivo* growth of KRAS-G12C-expressing tumours. Given the dependence of ARS-853 on nucleotide cycling and GTPase activity, it would be expected that additional KRAS mutations affecting these processes could promote resistance to ARS-853 in KRAS-G12C-expressing cells.

Lydia Shipman

> “KRAS-G12C is able to cycle between GDP- and GTP-bound states and ... ARS-853 inhibits this cycling”

ORIGINAL ARTICLES Lito, P. *et al.* Allele-specific inhibitors inactivate mutant KRAS G12C by a trapping mechanism. *Science* http://dx.doi.org/10.1126/science.aad6204 (2016) | Patricelli, M. P., Janes, M. R. *et al.* Selective inhibition of oncogenic KRAS output with small molecules targeting the inactive state. *Cancer Discov.* http://dx.doi.org/10.1158/2159-8290.CD-15-1105 (2016)