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KRAS INHIBITORS

Hitting the hotspots

Despite well-established tumorigenic roles of KRAS mutants, targeting their smooth surfaces was a challenge, which was overcome through the development of G12C-specific covalent inhibitors. A new study shows that optimizing non-covalent interactions with a cryptic pocket produces remarkable potency for another hotspot mutation.

Christopher B. Marshall and Mitsuhiko Ikura

Activating mutations in the small GTPase KRAS contribute to tumorigenesis in at least 15% of all human cancers;[1] however, the development of inhibitors of mutant KRAS proteins has been notoriously challenging due to an apparent lack of druggable pockets. Recently, mutant-specific covalent inhibitors have been developed for the variant with replacement of the glycine at position 12 with cysteine (G12C), which represents 12% of cancer-associated KRAS mutations[1]. These inhibitors interact with a shallow pocket near a loop called 'switch-II' (SIIP) of the KRAS protein and induce opening of this docking site, then irreversibly target the thiol introduced by the G12C substitution. In this issue of *Nature Chemical Biology*, Vasta et al. show that some of these inhibitors also interact non-covalently with the 'hidden' SIIP of other common KRAS mutants in vitro and in live cells to inhibit oncogenic signaling[2].

The SIIP (Fig. 1) was identified by crystallization of KRAS with a screening hit covalently adducted to Cys12 (ref. [3]). Consistent with the flexibility of SIIP residues, this highly plastic pocket has been shown to accommodate chemically diverse inhibitors, such as AMG510, ARS 1620 and MRTX849[4]. These covalent inhibitors specifically target the inactive GDP-bound state of KRAS(G12C), with the mutant undergoing sufficient nucleotide cycling to enable inhibitor engagement of KRAS-GDP[4].

MRTX849 was previously reported to have high non-covalent affinity for the SIIP[5], but it was not clear this was applicable to other KRAS mutants. Indeed, using nuclear magnetic resonance (NMR), Vasta et al. confirmed that the KRAS(G12C) covalent inhibitors MRTX849 and MRTX1257 exhibited non-covalent affinity for the SIIP of GDP-bound wild-type KRAS and KRAS(G12D), but not their GTP-bound forms[2]. In contrast, the first clinically approved KRAS(G12C) inhibitor, AMG510, exhibited much weaker affinity for wild-type KRAS-GDP, which indicates it is more dependent on covalent attachment. NMR

a

SI/IIP binders (non-covalent)
BI-2852
G12D (6GJ8)
DCAI
G12D (4DST)
Vanderbilt 13
G12D (4EPY)
Novartis Cmpd2
G12V (6CCX)

GTP (analog)
Switch I
Mg^{2+}
Cys12
SI/IIP (P1)
MRTX849
SIIP
BI-2852
Switch II

SIIP covalent
AMG510 (sotorasib)
G12C (6OIM)
ARS1620
G12C (5V9U)
MRTX849 (adagrasib)
G12C (6UT0)
MRTX1257
G12C

SIIP non-covalent
MRTX1133
G12D (7RPZ)
MRTX-EX185
G12D

b

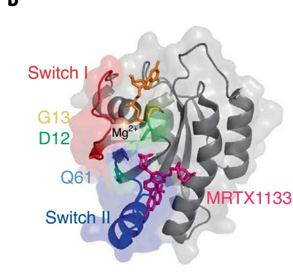

Fig. 1 | Flexible switch I/II and switch II pockets targeted in KRAS. a, Structure of GDP-bound KRAS(G12C) tethered to the covalent inhibitor MRTX849 (Protein Data Bank (PDB) accession code, 6UT0; pink), overlaid with KRAS(G12D) bound to a GTP analog in complex with BI-2852 (PDB, 6GJ8; light blue). The switch I and switch II regions (labeled) are shaded darker, and an arrow traces the structural transition of switch II between the two structures. MRTX849 is bright pink, BI-2852 is green, and the GTP analog is orange (GDP overlaps the analog and is not shown). Mg^{2+} is shown as a sphere. The switch I/II (SI/IIP) and switch II (SIIP) pockets are labeled, and selected inhibitors that interact with each site are listed in the tan text boxes (PDB accession codes in parentheses, where available). Note that the BI-2852 moiety that projects away from KRAS interacts with a second KRAS protomer (not shown). **b**, Structure of GDP-bound KRAS(G12D) in a non-covalent complex with MRTX1133 (PDB, 7RPZ), illustrating the proximity of the inhibitor to hotspot KRAS mutation sites. Switch I is red; switch II is blue; MRTX1133 pink; and GDP is orange, with Mg^{2+} shown as a sphere. Oncogenic mutation hotspots are indicated (D12, green; G13, yellow; Q61, cyan).

analysis of a patent compound from Mirati (MRTX-EX185) that was known to inhibit KRAS(G12D) revealed that MRTX-EX185 interacted non-covalently with GDP-bound wild-type KRAS and KRAS(G12D), as well as the GTP-bound forms of both proteins, albeit with lower affinity[2]. The recent structure of an MRTX-EX185 analog, MRTX1133 (Fig. 1b), in complex with KRAS(G12D)-GDP revealed the details of its high-affinity interaction with the SIIP[6].

To confirm their NMR findings in vivo, Vasta et al. developed an assay based on bioluminescence resonance energy transfer (BRET), using luminescent NanoBiT fused to KRAS as the BRET donor and a fluorophore conjugated to the Boehringer-Ingelheim KRAS inhibitor BI-2852 as the acceptor[2]. Binding of the inhibitor to SIIP was expected to displace the acceptor and diminish the BRET signal. After validation with covalent inhibition of KRAS(G12C), the assay revealed that MRTX849 and MRTX1257 were able to engage wild-type KRAS and other oncogenic mutants (KRAS(G12D) and KRAS(Q61H/L), but not KRAS(G12V) or KRAS(Q61R)). Remarkably, MRTX-EX185 engaged KRAS(G12D) with high potency (half-maximal inhibitory concentration, 90 nM), and inhibited binding of the kinase CRAF, signaling via the kinase MAPK and proliferation[2]. Mutants with other hot-spot substitutions (G12C/V, G13D and Q61H/L/R) were also engaged by MRTX-EX185, albeit less tightly.

Together, the BRET and NMR data indicate that SIIP can be engaged without covalency and indicate a role for engaging KRAS-GTP, which will pave a new path for inhibitors of non-Cys KRAS mutants. Although oncogenic KRAS mutations

promote the GTP-bound state, each has distinct biochemical properties that control the GTPase cycle[7] and accessibility of KRAS-GDP, which highlights the importance of targeting both states for slowly cycling mutants.

The success of high-affinity non-covalent SIIP inhibitors represents a major advance that provides optimism for structure-guided therapeutics that target other KRAS mutants and RAS isoforms. The proximity of the three hot-spot mutation sites (Gly12, Gly13 and Gln61) to SIIP (Fig. 1b) suggests potential for specific interactions with mutant residues.

Notably, the other major KRAS pocket, the 'switch I/II pocket' where BI-2852 binds (Fig. 1a), is also 'cryptic' and induced by small-molecule binding; however, this site is not GDP or GTP specific[8]. These flexible pockets highlight the importance of understanding the role of KRAS dynamics in 'induced fit' (or 'conformational selection'). Interestingly, binding of an inhibitor to KRAS SIIP displaced BI-2852[2], despite non-overlapping binding sites, which suggests dynamic allosteric connections between the two sites. Understanding the allosteric interplay between these transient KRAS pockets in the GDP- and/or GTP-bound state(s) will contribute to inhibitor optimization.

It is now clear that RAS proteins are no longer undruggable. However, challenges continue, as acquired resistance to covalent G12C inhibitors has emerged rapidly in the clinic, including alterations of SIIP residues and secondary mutations of Cys12[9,10]. Non-covalent SIIP inhibitors may be effective against secondary C12X mutants, but they will almost certainly be evaded by SIIP mutations. Some of the AMG510- and MRTX849-resistant mutations are mutually exclusive[9,10], which suggests that the availability of multiple scaffolds may provide opportunities to overcome the acquired resistance of some SIIP mutations, while compounds that target the other switch I/II pocket might more comprehensively overcome SIIP-mutant resistance. ❐

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Competing interests
The authors declare no competing interests.