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Cancer: Drug for an undruggable protein

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Scientists have long aimed to develop drugs against the cancer-associated protein KRAS, but without success. An approach that targets the oncoprotein's cellular localization reignites lost enthusiasm. **See Letter p.638**

Human RAS genes have two claims to notoriety. First, they make up the most frequently mutated oncogene family in human cancer, having a prevalence of one in every three cases¹. Second, despite more than three decades of intensive effort, no effective pharmacological inhibitor of the RAS oncoprotein has reached the clinic. So it is exciting that, on page 638 of this issue, Zimmermann et al.² report the identification and characterization of a small-molecule inhibitor that interferes with the localization of KRAS - the RAS isoform most commonly mutated in human cancers - to the plasma membrane surrounding cells³.

Following their synthesis in the cytoplasm, RAS proteins are initially inactive⁴. They then undergo a series of rapid post-translational modifications that ensure their association with the inner leaflet of the plasma membrane, where these proteins exert their normal, as well as their cancer-associated, signaling activity. Therefore, most efforts aimed at anti-RAS drug discovery have involved indirect approaches to block the activities of proteins that either promote plasma-membrane association of RAS or are components of its downstream signaling pathway.

The key post-translational modification of RAS involves the addition of a 15-carbon farnesyl lipid tail in a reaction catalysed by the farnesyltransferase enzyme. This modification facilitates RAS association with membranes and is essential for proper RAS localization and activity, having prompted intensive efforts in the 1990s to develop farnesyltransferase inhibitors (FTIs).

Despite promising results in preclinical studies, however, the results of clinical trials with FTIs were disappointing. The inhibitors blocked membrane association of the HRAS isoform, but lacked antitumour activity in cancers involving mutated KRAS (and NRAS). KRAS could still associate with the plasma membrane through an unexpected compensatory activity of the farnesyltransferase-related enzyme geranylgeranyltransferase-I, which modifies RAS with a geranylgeranyl, rather than a farnesyl, group. This discouraging outcome greatly dampened interest in targeting RAS - and, in particular, its membrane

association - for cancer treatment. Instead, ongoing efforts have mainly focused on inhibitors of the RAF-MEK-ERK and the PI3K-AKT signalling cascades downstream of RAS.

Zimmermann et al. describe an approach aimed at disrupting KRAS membrane association that warrants reassessment of the current strategies. The authors identify and characterize a small-molecule inhibitor of PDE δ , a protein that can bind to and regulate the trafficking of RAS and RAS-related proteins to membrane compartments⁵⁻⁸ (Box 1). Specifically, PDE δ contains a deep, hydrophobic pocket capable of binding the lipid moiety of farnesylated proteins, in particular RAS.

An earlier study⁸ found that suppression of PDE δ levels disrupts RAS association with the plasma membrane and impairs the growth of RAS-mutant cancer cells. This finding prompted Zimmermann et al. to perform a high-throughput screen to identify small molecules that could block PDE δ association with the farnesylated tail of KRAS. After identifying several hits, they took a structure-based drug design approach to develop their most promising compound, designated deltarasin.

The researchers' fluorescence microscopy experiments validate deltarasin's ability to block PDE δ -KRAS interaction in live cells. Following addition of 5-micromolar deltarasin to human KRAS-mutant pancreatic-cancer cell lines, PDE δ could no longer redistribute KRAS to the plasma membrane. Deltarasin also impaired the proliferative capacity of the pancreatic-cancer cell lines, which depends on signalling of mutant KRAS. Furthermore, it greatly reduced KRAS-dependent signalling events, such as phosphorylation of the ERK1 and ERK2 proteins. When the authors assessed the effects of deltarasin in vivo, in a mouse model of pancreatic ductal adenocarcinoma, they observed a dose-dependent reduction in tumour growth.

Recently, there has been a resurgence of interest in targeting RAS. This is due in part to the findings of cancer-genomics studies, which have reaffirmed KRAS mutations as the predominant oncogenic abnormalities in several cancers, including pancreatic, lung and colorectal cancers. However, the task of blocking mutant KRAS function with anticancer drugs remains daunting, requiring identification of fresh strategies. Zimmermann and colleagues' finding, that pharmacologically disrupting the ability of PDE δ to promote plasma-membrane association of KRAS impairs the growth of KRAS-mutant pancreatic tumour cells, points to a provocative and innovative approach that may succeed where FTIs failed (Box 1).

Indeed, the efficacy of a PDE δ inhibitor will not be circumvented by alternative prenylation mechanisms, such as geranylgeranylation, that prevent FTIs from blocking KRAS (and NRAS) association with the plasma membrane. Nonetheless, as is the case with FTIs, inhibition of RAS regulation by PDE δ will probably have unforeseen consequences. PDE δ can interact with other farnesylated proteins, including farnesylated RAS-family proteins that act as tumour suppressors (for example, NOEY2, also called DiRAS3). Inhibition of such beneficial proteins may lead to toxic effects in normal cells.

There are also conflicting observations regarding PDE δ selectivity for farnesylated and geranylgeranylated proteins⁶. If PDE δ is also required for the trafficking of

geranylgeranylated proteins (such as Rho proteins), then additional off-target effects may be seen.

Another unresolved issue is exactly how dependent RAS proteins are on PDE δ for proper localization, because even in the absence of PDE δ , KRAS can bind to cell membranes⁸. That KRAS deficiency is lethal, whereas PDE δ deficiency is not⁹, underscores the fact that at least some KRAS functions are PDE δ independent. So, although Zimmermann and co-authors' data are exciting, much remains to be learnt about PDE δ function and mutant-RAS dependency on it for proper subcellular localization.

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Box 1**Inhibitors of RAS signaling pathway**

After their synthesis, inactive RAS proteins undergo a rapid series of post-translational modifications at a CaaX tetrapeptide motif on their carboxy terminus (C denotes the amino acid cysteine; aa, two saturated hydrocarbon (aliphatic) residues; and X, a variable residue). The enzymes that mediate this modification include farnesyltransferase (FTase), which attaches a 15-carbon farnesyl lipid to RAS. Other modifications to this lipid and to the CaaX motif follow, before the PDE δ protein is attached to the farnesylated tail of RAS, thereby facilitating RAS accumulation at the plasma membrane.

Pharmacological strategies to prevent activated RAS from reaching the plasma membrane include farnesyltransferase inhibitors such as lonafarnib and tipifarnib. Both of these are considered to be ineffective against KRAS and NRAS, however, because they induce alternative modification of RAS proteins by geranylgeranyltransferase-I (GGTase-I). Lonafarnib is under clinical evaluation for treating the genetic disorder progeria, and is showing highly promising results. Another agent, salirasib, is a farnesyl mimic that competes with active RAS for binding to the oncoprotein's docking protein, galectin, on the plasma membrane, leading to degradation of active cytoplasmic RAS. Salirasib has shown promising results in a clinical trial for pancreatic cancer¹⁰. Zimmermann et al.² describe a new drug candidate, deltarasin, which inhibits PDE δ binding to farnesylated RAS.

In addition to these inhibitors of Ras association with the plasma membrane, more than 20 inhibitors that target the RAF-MEK-ERK signalling cascade downstream of RAS are under clinical evaluation. Of these, vemurafenib, a RAF inhibitor, has been approved for use in patients with BRAFmutant metastatic melanoma, a skin cancer. **N.M.B. & C.J.D.**