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DRUG DISCOVERY

Pocket of opportunity

After three decades of unsuccessful efforts to develop small molecules that neutralize the cancer-causing Ras proteins, an approach has been found that opens up fresh avenues for anticancer research.

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One-third of all tumours harbour mutations in *RAS* genes[1], but the Ras proteins encoded by these mutant genes have steadfastly eluded targeting by therapeutic agents. In a paper published on *Nature*'s website today, Ostrem *et al.*[2] present perhaps the most promising strategy ever pursued towards developing an anticancer drug that targets mutant Ras proteins. The authors' clever approach was to make compounds that affect a subset of Ras mutations in which a particular amino acid — glycine-12 — in the protein is replaced by another amino acid, cysteine. This kind of mutation, dubbed G12C, is found in a substantial proportion of lung cancers. Because the G12C mutation exists only in tumour cells, drugs that target it could be exquisitely selective, and therefore potentially much less toxic than many current anticancer drugs.

Normal cellular Ras is a small protein that serves as a switch for cell signalling[1]. It binds the nucleotide GTP, hydrolysing it to form another nucleotide, GDP, and so cycles between GTP-bound 'on' and GDP-bound 'off' states. Mutations such as G12C impair GTP hydrolysis and trap Ras in the GTP-bound 'on' state, causing unregulated signalling that can lead to cancer. In the human body, Ras is therefore both friend and foe: the non-mutated protein is the beating heart of cell signalling, but mutated versions are the villainous masterminds of malignancies.

No drugs that combat Ras-driven human cancers have so far been developed, and the stakes for doing so are high: we cannot win the war on cancer without taming Ras. Accordingly, the US National Cancer Institute this year allocated US$10 million specifically to develop such drugs, and key researchers in the field have committed to making this effort a reality[3].

In fact, medicinal chemists have long attempted to halt unregulated Ras, but were unable to identify small molecules that could access the nucleotide-binding pocket in Ras in order to do so. Drug development at large pharmaceutical companies had therefore been focused on inactivating Ras indirectly by cropping its lipid tail — a feature that it uses to attach itself to cell membranes. This led to the discovery of compounds known as farnesyltransferase inhibitors, which stall an enzyme that is involved in attaching the lipid tail to Ras. But although these compounds were active in animal models, they were ineffective in human patients with cancer, because cancer cells replaced the cropped tail with an alternative one[4]. Earlier this year, biochemists discovered another target[5] to prevent the membrane localization of Ras: a protein called PDEδ. The first PDEδ blockers to be developed inhibit the cancer-causing activity of mutant Ras, but an anxious wait is in store before we know whether this approach will yield effective anticancer drugs.

With recent improvements in drug design guided by protein structures, there is renewed interest in targeting Ras directly. The latest generation of drug developers has thus replaced the previous sledgehammer approach with one that has scalpel-like precision. Within a year, three groups have reported small molecules that directly modulate Ras activity[6–8]. However, the compounds bind weakly to the protein, because the targeted binding pockets are shallow, and it is unclear whether binding affinity can be improved sufficiently for anticancer applications.

Ostrem *et al.* now report a first volley of Ras inhibitors that work by attacking the cysteine residue of the Ras G12C mutation by means of a thiol (SH) group, forming a disulphide (S–S) bond to the residue's side chain and so providing a foothold on Ras. When the authors obtained an X-ray crystal structure of an inhibitor-tethered protein, they observed a newly exposed pocket that presents new opportunities for drug discovery (Fig. 1). Unfortunately, thiol-based inhibitors are not very 'drug-like' because they are rapidly degraded in cells. However, the researchers have begun to optimize their prototype compounds and have made improved versions carrying 'warheads' that bind irreversibly to cysteine. These compounds are appropriate for biochemical and cellular studies.

Interestingly, Ostrem and colleagues' inhibitors have a preference for the GDP-bound form of Ras, and the authors' biochemical assays show that the compounds prevent the mutant protein from binding GTP. This is desirable behaviour for an anticancer drug because it traps inactive Ras, interrupting signalling through the downstream effectors that cause cancer. Indeed, the researchers find that their partly optimized compounds partially block Ras signalling in cells and exhibit some selectivity for G12C-expressing cells, blocking their proliferation in preference to that of cells that lack a G12C mutation. This selective antiproliferative activity

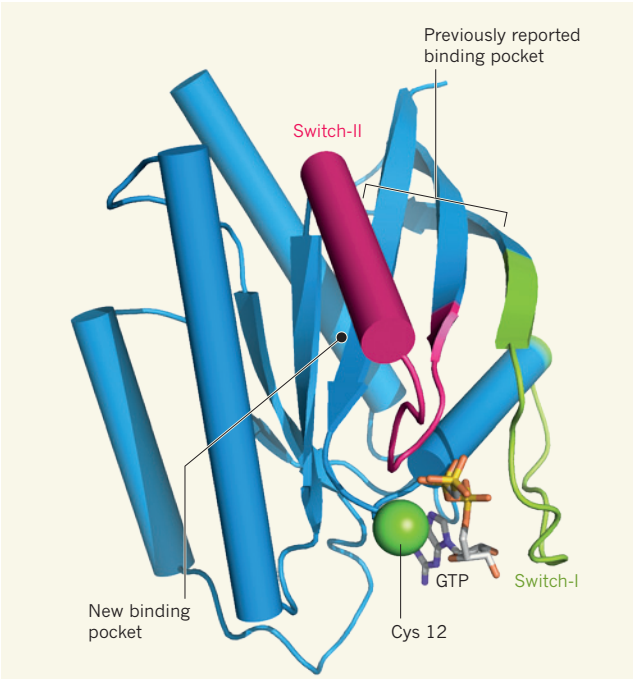

Figure 1 | Binding pockets for Ras inhibitors. In the G12C cancer-causing mutant of the protein Ras, depicted here as a ribbon diagram, a cysteine amino acid (Cys 12) replaces a glycine. Cylindrical sections indicate α-helices; ribbons indicate β-sheets. The protein's substrate, the nucleotide GTP, is bound at the bottom right. Previously discovered Ras inhibitors[5–7] bind to a region between switch-I and switch-II, which are the main regions of Ras that interact with regulators and effector molecules. Ostrem *et al.*[2] have discovered inhibitors that bind irreversibly to Cys 12. This led to the identification of a new binding pocket, which in turn allowed the authors to prepare Ras inhibitors of increased potency.

validates the authors' approach and suggests that a truly effective drug might be possible if the compounds can be improved further.

It should be emphasized that even the best of the reported compounds are not suitable for use as drugs. The compounds do not completely block Ras signalling in cells, and it is unclear whether this is a limitation of the overall approach or of the incompletely optimized compounds. Although there are grounds to hope that drugs could be developed that have a substantial therapeutic index (that is, being highly effective with minimal toxicity), there may be a limit to the balance that can be achieved between efficacy and side effects. Moreover, bringing a successful drug to the market will require substantial further investment.

Another caveat concerning Ostrem and colleagues' strategy is that compounds that form irreversible bonds with cysteine are inherently reactive. This might be a problem, because reactive compounds tend to be toxic. However, the idea of anticancer agents that work by irreversibly binding cysteine has gained considerable traction with the recent successes of the drugs afatinib[9] and ibrutinib[10], which act in this way. Nevertheless, Ostrem *et al*. are attempting to further exploit the G12C foothold by designing compounds that no longer require irreversible binding to cysteine, which might lead to compounds that are active against other Ras mutations. This would broaden the population of patients who could benefit from such compounds, but may come at the expense of the drugs' therapeutic index. ■

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