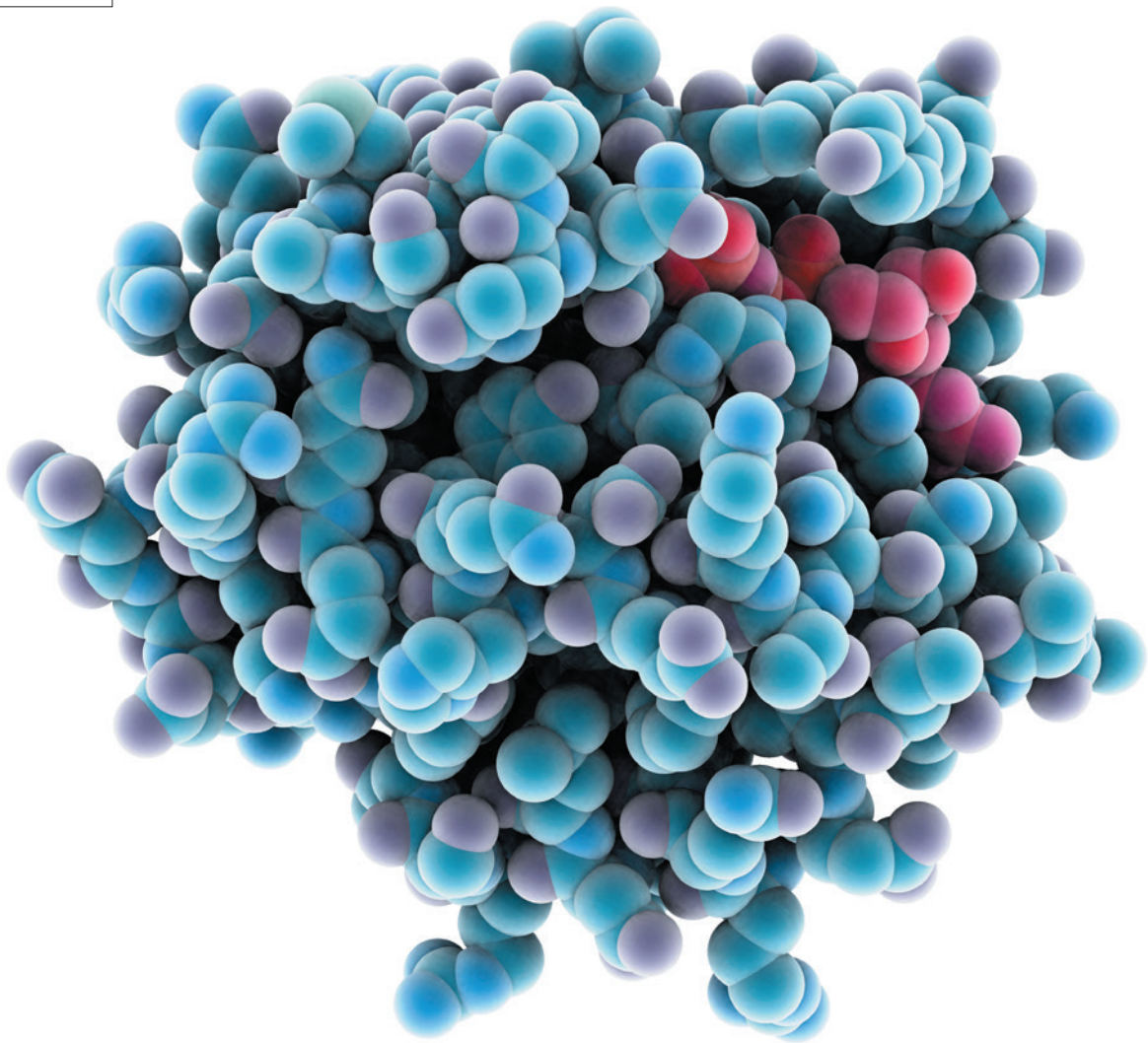

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THE RAS RENAISSANCE

Thirty years of pursuit have failed to yield a drug to take on one of the deadliest families of cancer-causing proteins. Now some researchers are taking another shot.

BY HEIDI LEDFORD

When Stephen Fesik left the pharmaceutical industry to launch an academic drug-discovery laboratory, he drew up a wanted list of five of the most important cancer-causing proteins known to science. These proteins drive tumour growth but have proved to be a nightmare for drug developers: they are too smooth, too floppy or otherwise too finicky for drugs to bind to and block. In the parlance of the field, they are 'undruggable'.

One of the first culprits that Fesik added to his list was a protein family called Ras. For more than 30 years, it has been known that mutations in the genes that encode Ras proteins are among the most powerful cancer drivers. Ras mutations are found in some of the most aggressive and deadly cancers, including up to 25% of lung tumours and about 90% of pancreatic tumours. And for some advanced

cancers, tumours with Ras mutations are associated with earlier deaths than tumours without them.

Decades of research have yet to yield a drug that can safely curb Ras activity. Past failures have driven researchers from the field and forced pharmaceutical companies to abandon advanced projects. But Fesik's laboratory at Vanderbilt University in Nashville, Tennessee, and a handful of other teams have set their sights anew on the proteins. They are armed with improved technology and a better understanding of how Ras proteins work. Last year, the US National Cancer Institute launched the Ras Initiative, a US$10-million-a-year effort to find new ways to tackle Ras-driven cancers. And researchers are already uncovering compounds that, with tweaking, could eventually yield the first drugs to target Ras proteins.

Researchers are mindful that they still have many hurdles to jump. "You have to have a lot of respect for Ras," says Troy Wilson, president of Wellspring Biosciences, a company in La Jolla, California, that launched in 2012 with its sights set on Ras. "It is not to be underestimated. But it's also one of the most important oncogenes in cancer."

Advocates of this Ras renaissance say that any signs of success could provide lessons on how to target other important proteins that are deemed to be undruggable. Just because people assume Ras proteins are too difficult to target does not mean that scientists should give up, says Channing Der, a cancer researcher at the University of North Carolina at Chapel Hill. "Dogma is a moving target."

HIGH-HANGING FRUIT

In 1982, Der's team was one of the first to show that mutations in human genes encoding Ras proteins can cause cancer[1]. This finding marked the culmination of a hunt for oncogenes — genes that can drive cancer — in the human genome. They had previously only been described in viruses and animal models.

The discovery laid the foundation for the modern cancer-research juggernaut, with its emphasis on tracking genetic mutations and mapping altered molecular pathways. It also prompted hopes of finding drugs that would target oncogenes and cure some cancers.

The following years were filled with discovery. It became clear that humans produce three highly similar Ras proteins and that these are activated when cells need to proliferate (to replace damaged tissue, for example). Signals from outside the cell switch Ras to an 'on' state, in which it is bound to a molecule called GTP. Cancer-causing forms of Ras proteins have a disabled 'off' switch and cannot properly process the GTP. So it seemed logical to search for drugs that could interfere with GTP binding to stop mutant Ras.

But as the understanding of Ras biochemistry grew, so too did a sense of pessimism. The family's affinity for GTP turned out to be extraordinarily high, and finding another compound that could block GTP's access seemed impossible. Ras proteins also work by interacting with other proteins, but small-molecule drugs that are able to get inside cells are often too small to cordon off the wide surface area usually involved in protein–protein interactions. (Antibodies can make excellent drugs and can mask a large area on their targets, but most do not penetrate cell membranes.)

> "PEOPLE SAID, 'NOBODY HAS DONE ANYTHING IN THE FIELD FOR TEN YEARS. LET'S DO SOMETHING'."

Ras structures offered more reasons for concern. Drug developers look at a protein's shape to gauge the likelihood of finding a compound that will bind to a critical site. They like to see a protein with deep pockets that a drug can slip into and bind with multiple points of contact. However, Ras proteins are relatively smooth.

Twenty years ago, researchers thought they had the problem solved. To function, Ras proteins need to latch on to the inside of the cell membrane through a fatty tail. That tail is added by farnesyl transferase — an enzyme that is more amenable to drug targeting than Ras proteins. So the idea was to hobble Ras activity by finding drugs that inhibit farnesyl transferase.

At first, it looked like a winning strategy. Farnesyl transferase inhibitors damped down cell proliferation in mice and human cancer cells[2]. By the early 2000s, at least six pharmaceutical companies were racing to bring the drugs to market. Many abandoned other Ras-related projects because they thought the Ras problem was solved, says chemist Herbert Waldmann of the Max Planck Institute of Molecular Physiology in Dortmund, Germany. "The whole field took a deep breath and waited," he says.

The wait ended with one of the biggest disappointments in pharmaceutical history. One by one, the drugs failed in human clinical trials. Der, who was still studying Ras at the time, says that the episode taught him, and everyone else, an important lesson about Ras biology.

The three forms of human Ras are nearly identical in terms of structure and amino-acid sequence. Researchers assumed that their functions would be similar too. Most of the tools used to study Ras proteins — cell cultures, transgenic mice and antibodies — were developed using H-Ras, which was easier to work with than the other forms. "All of us, including myself, thought why bother studying the other ones when we can just learn all about H-Ras," says Der. "Unfortunately, a lot of money was spent on that misconception."

It turned out that the other two forms of Ras in humans — K-Ras and N-Ras — are much more important in cancer, and the cell has a contingency plan in place to keep them working. In the absence of a farnesyl tail, another enzyme is able to tack on a different fatty tail, rendering the experimental drugs useless.

The Ras field was scarred by this episode, and it took some time before researchers were willing to give the proteins another look. But about a decade later, they started coming back. "All of a sudden people turned around and said, 'Hey, this is still one of the most important targets in oncology. Nobody has done anything in the field for ten years. Let's do something,'" says Waldmann. This time, researchers took a fresh approach by looking for weaknesses in Ras-driven tumours.

One such weakness is 'synthetic lethality'. When Ras proteins are in overdrive, cancer cells often become dependent on other molecular pathways for survival. Blocking these other pathways might not affect normal cells, but it kills Ras-driven tumour cells. Laboratories set about screening for the synthetic-lethal partners of mutated genes encoding Ras, with the idea that targeting them would kill cancer cells but leave normal cells unaffected.

The result was a wave of papers reporting possible new targets — followed closely by another wave of reports that the synthetic-lethal results were irreproducible[3]. Last October, William Sellers, Global Head of Oncology at the Swiss drug maker Novartis, reported at a conference that his team had tried and failed to reproduce the most prominent published Ras synthetic-lethal findings. Changes in context, such as the cell type used or specific screening conditions, could easily change the outcome of the experiment, says Julian Downward, a cancer researcher at the Francis Crick Institute in London. Researchers are still sifting through the results to find targets that hold up, but Downward is doubtful that the efforts will bear fruit. "Everyone seems to get something different from those experiments," he says. "I suspect these are not going to be the most robust targets."

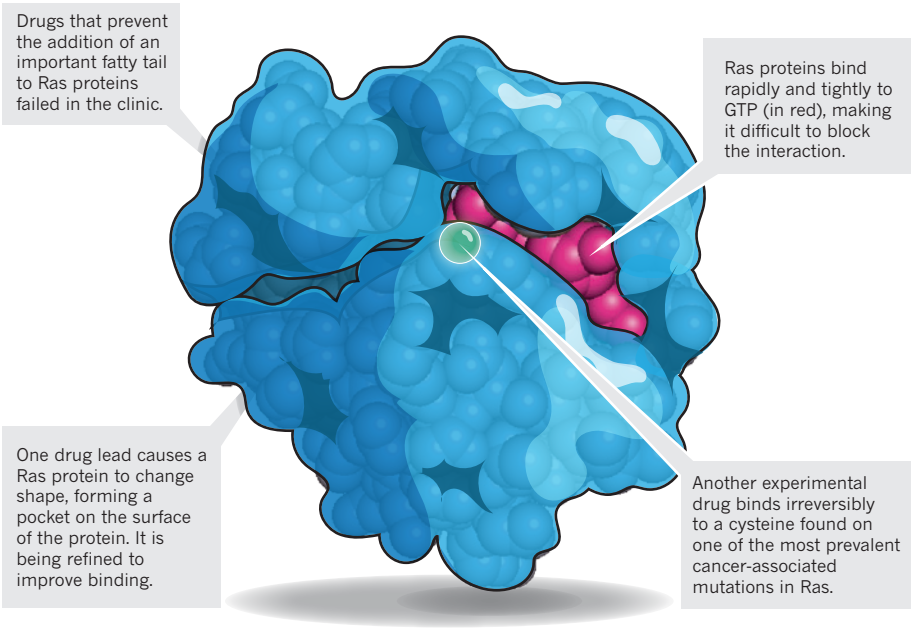
TAILORED TO FIT

With the disappointment of the synthetic-lethal approach fresh in their minds, several researchers have been looking to target Ras itself (see 'Ras attack'). "We decided you have to go to Ras directly," says Brent Stockwell, a chemical biologist at Columbia University in New York.

Improvements made during the past five years in computer modelling and in ways of screening for drug compounds offer fresh hope for targeting the smooth, unpocketed terrain

RAS ATTACK

Ras proteins have proved devilishly hard to make drugs against. They have a relatively smooth surface with few pockets where a molecule might bind tightly.



of Ras proteins, Stockwell says. Researchers are now better able to predict the affinity of small molecules for proteins, for example, and have a better understanding of protein dynamics.

Stockwell's team is capitalizing on this to design small molecules that are tailored to the surface of Ras proteins — first in the computer, and then in the laboratory. "Maybe for these proteins, you're just not going to find the right solution anywhere out there in the world," Stockwell says. "You've just got to make it."

Fesik is also building new drugs, but starting from a library of existing compounds. In his former career at Abbott Laboratories in Abbott Park, Illinois, Fesik devised ways to disrupt interactions between proteins by piecing together fragments of compounds that bind, however weakly, to the target. The result is a large, novel compound that is unlikely to be found in the standard chemical libraries used to hunt for drugs.

Fesik likens the technique, called fragment-based screening, to constructing a key to fit a lock by cutting one notch at a time. "Eventually you combine all the notches," he says. "The compound has never been made before and yet you find it because you're building it up slowly and tailoring it to your protein."

Fesik's lab and his industry collaborators have found more than 130 molecules that bind weakly to K-Ras[4]. The compounds induce a change in the protein's structure, opening up a binding pocket in the process. The team is now trying to add on other fragments to improve the fit — in effect, the second notch in the key. Der notes that Fesik built a reputation for drugging the undruggable in industry before he left to pursue an academic career. "If anyone is going to do it, it is Fesik," he says.

Others are looking more closely at exploiting specific mutations within K-Ras. Although there are many different cancer-associated mutations in the gene that encodes it, just three are responsible for the vast majority of Ras-driven cancers. Each of these yields an enzyme with slightly different behaviour, says Der. "If we begin to think about different mutations as having different personalities, those different personalities may open up unique vulnerabilities," he says.

Kevan Shokat, a chemical biologist at the University of California, San Francisco, joined the Ras hunt six years ago. In 2013, he reported a compound that targets a K-Ras mutation known as G12C (ref. 5). The mutation, which is found in 20% of lung cancers, replaces the amino acid glycine with cysteine, which readily reacts with other molecules. Shokat's compound exploits the reactive cysteine and binds to it irreversibly. The inhibitor will require additional tinkering before it can be used in human patients but, as the first drug candidate that truly binds directly to Ras, it has generated a tremendous amount of excitement, says Downward. "It has re-energized the whole area," he says.

Shokat says he has long thought that a mutation-specific approach might work, but he hesitated to pursue it in his laboratory until recently. Drug developers were afraid of drugs that seize upon their target and do not come off, he says, because they seemed more likely to have unanticipated reactions with other proteins in the body. But several successful drugs, such as the lymphoma and myeloma drug ibrutinib, have recently been found to bind irreversibly to their targets.

Meanwhile, pharmaceutical companies are increasingly open to the idea of developing drugs that work in subsets of patients with cancer who carry specific mutations. "There won't be one drug that will work for every K-Ras patient," predicts Timothy Burns, a cancer researcher at the University of Pittsburgh in Pennsylvania.

Fesik says that the solutions to Ras's puzzles, whatever they are, will probably emerge from academic institutions. He left pharma in part because he loved the pursuit of important targets, regardless of how easy or hard they are to hit. Chasing an undruggable protein can be difficult to justify in industry, where scientific interest must often take a backseat to the near-term potential for profit. "Most pharma companies don't want to take the risk to go after these undruggable targets, and if they do, it's temporary," he says.

Bridges are forming, however. Fesik's laboratory has partnered with the German pharmaceutical company Boehringer Ingelheim to evaluate its first-generation Ras-binding drug. And Shokat co-founded Wellspring Biosciences to bring his inhibitor to market. The work soon won support from Janssen Biotech of Horsham, Pennsylvania.

The efforts are getting government attention as well. The multimillion-dollar Ras Initiative is supporting the development of tools and basic research on Ras protein structures to aid drug discovery, says Frank McCormick, a cancer researcher at the University of California in San Francisco and co-director of the project. "We are trying to de-risk Ras as a target so that others will jump back in the ring and have another shot," he says.

For years, the pharmaceutical industry has pursued low-hanging fruit in a different category of proteins called kinases, McCormick says. Those were easier to target, and yielded many useful cancer drugs. But that wave is starting to subside, he argues, and it is time to focus on the higher-hanging fruit: tougher targets, such as Ras proteins, that are known to be crucially important.

Stockwell says he hopes that the recent revival of research on Ras proteins could inspire scientists studying other intractable targets. "If there is some success there, maybe that excitement will extend to other targets," he says. "If we really want to impact disease, there's this vast space of additional targets that have never been mined." ■

Heidi Ledford *writes for* Nature *from Cambridge, Massachusetts.*

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