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Interfacing with Ras

By Amy Donner, Senior Editor

A German team has identified a compound that disrupts a protein-protein interaction that localizes K-Ras to the cell membrane, thus inhibiting tumor growth.[1] The interface provides a new small molecule binding site for the handful of companies and academics working on ways to tackle the previously undruggable Ras family.

Ras proteins are molecular switches that control cell growth and proliferation. Mutations and other mechanisms that aberrantly activate Ras signaling can promote tumorigenesis.

Collectively, mutations in genes encoding the three Ras isoforms—K-Ras, v-Ha-ras Harvey rat sarcoma viral oncogene homolog (HRAS) or neuroblastoma Ras viral (v-Ras) oncogene (NRAS)—occur in nearly 20% of all cancers.

K-Ras is by far the most commonly mutated isoform, with activating mutations occurring in about 80% of pancreatic cancers.

"If one is diagnosed with pancreatic cancer based on a mutation in *K-Ras*, the life expectancy is months. And there is hardly anything the doctors can do," said Herbert Waldmann, professor of chemistry and managing director at the **Max Planck Institute of Molecular Physiology**.

"K-Ras is a particularly important driver in pancreatic cancer. This is essentially an untreatable cancer—there is a huge unmet clinical need," added John Hancock, integrative biology and pharmacology chair and professor at **The University of Texas Health Science Center at Houston**.

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>
> *—Thomas Hegendörfer, Lead Discovery Center GmbH*

Until 2012, nobody had been able to target any form of Ras with a small molecule because the proteins lacked well-defined surface pockets suitable for binding drug molecules (*see* **Box 1, "A complex question"**).

Last year, independent teams at **Roche**'s **Genentech Inc.** unit and the

Box 1. A complex question.

Whereas German researchers have uncovered a way to target K-Ras, a team from Texas has shown that a prior approach of disrupting Ras interactions with rho guanine nucleotide exchange factors (ARHGEFs; GEFs) may be more clinically relevant than previously believed.

GEFs such as son of sevenless homolog 1 (SOS1) catalyze the rate-limiting step in Ras activation. They promote the dissociation of GDP so GTP can bind and reactivate Ras. Two groups, one from **Roche**'s **Genentech Inc.** unit and the other from the **Vanderbilt University School of Medicine**, independently reported compounds that act via Ras-GDP to disrupt the Ras-SOS1 interface and block Ras activation.

Nevertheless, other researchers had questioned whether blocking the Ras-GDP complex would be an effective therapeutic strategy because oncogenic Ras mutants are locked in a GTP-bound conformation, rendering them constitutively active. The expectation was that these mutants could be insensitive to small molecules that act via the GDP-bound conformation.

Now, John Hancock and Alemayehu Gorfe have shown that this is not exactly the case.[9]

Hancock is integrative biology and pharmacology chairman and professor at **The University of Texas Health Science Center at Houston**. Gorfe is assistant professor of integrative biology and pharmacology at the university.

In cells cultured for six hours, the team's inhibitors of Ras-GDP did not block oncogenic Ras signaling. However, when incubation periods were prolonged to three days, the compounds decreased oncogenic Ras signaling and oncogenic K-Ras–driven proliferation of multiple cancer cell lines compared with vehicle.

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According to Hancock, these prolonged assays demonstrate that "GTP turnover is slow—not absent—so exchange activity is still inhibited. Therefore, this study shows that blocking exchange factor interaction is a viable approach to inhibiting oncogenic mutant Ras function."

The research was published in the *Proceedings of the National Academy of Sciences*. The team also included scientists from the **University of Putra Malaysia**.

Hancock said, "It is very hard to predict which of the different approaches to inhibit Ras will be most successful. Inhibiting Ras at multiple levels will likely be the way to go. As we have learned from kinase inhibitors, we need to use two, possibly three drugs to achieve maximal inhibition at the lowest levels of toxicity."

Hancock and Gorfe declined to disclose the patent and licensing status of their work. *—AD*

Vanderbilt University School of Medicine identified small molecules that disrupted Ras, but the compounds were not potent and were not tested against oncogenic Ras.[2–4]

In April, a **Kobe University** team developed small molecule inhibitors of wild-type and oncogenic forms of Ras, but the compounds were far from drug-like in terms of potency.[5,6]

Now, a German team has overcome these shortcomings by targeting a distinct protein-protein interface that modulates oncogenic K-Ras activity.

K-Ras activation depends on its localization at the plasma membrane. Farnesyl protein transferases add a lipid modification called a prenyl group to the C-terminal end of K-Ras, which anchors K-Ras to the membrane. The phosphodiesterase δ subunit (PDEδ), a prenyl-binding protein, binds lipid-modified Ras and delivers it to the plasma membrane.[7]

With that mechanism in mind, Waldmann, Philippe Bastiaens and Alfred Wittinghofer led a structure-based initiative to identify small molecules that bind to PDEδ and disrupt its interaction with K-Ras. The goal was to delocalize K-Ras from the plasma membrane and inhibit its activity.

Bastiaens is professor of systemic cell biology at the Max Planck Institute of Molecular Physiology, and Wittinghofer is professor emeritus at the institute.

A high throughput screen identified small molecules that bound to the prenyl-binding pocket of PDEδ. The screen yielded several hits with a shared chemical scaffold. After validating these hits in secondary, *in vitro* PDEδ-binding assays, the scientists solved the crystal structure of PDEδ in complex with several compounds.

Using the cocrystal structures as guides, the team modified the initial hits to optimize interaction with and affinity for PDEδ. For cellular studies the group selected one compound—deltarasin—that bound PDEδ with a K_d of 38 nM *in vitro* but did not interact with off-target prenyl-binding proteins.

In cultured cells, deltarasin disrupted PDEδ-K-Ras interactions. Deltarasin delocalized K-Ras from the plasma membrane in pancreatic ductal adenocarcinoma cell lines. The molecule also produced dose-dependent decreases in oncogenic K-Ras–driven proliferation and signaling compared with vehicle in pancreatic cancer cell lines. In xenograft mouse models, deltarasin abrogated oncogenic K-Ras–driven pancreatic tumor growth.

Results were published in *Nature*. The team also included scientists from **Ruhr University Bochum**.

Building momentum against Ras

Waldmann said a drug discovery project based on deltarasin is ongoing at the **Lead Discovery Center GmbH**, which is the drug discovery arm of the **Max Planck Society**.[8]

"Within the next two years we are going to profile and optimize several compound series, including deltarasin and analogs, to generate a lead package," said Thomas Hegendörfer, head of business development at Lead Discovery Center.

Hegendörfer said projects usually start to attract interest from industry after showing efficacy in therapeutically relevant animal models. In the case of deltarasin, however, he said there are already pharma suitors.

One reason for the early interest, said Waldmann, is that deltarasin is more potent than other reported Ras inhibitors. "It also differentiates between wild-type- and K-Ras-dependent cell lines," he said. "The target is entirely novel."

The deltarasin compound class is patented by the Max Planck Society and will be available for licensing from **Max Planck Innovation GmbH**.

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