IN FOCUS

RAS at 40: Update from the RAS Initiative

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Summary: The RAS Initiative was launched in 2013 to address unmet clinical needs of patients with KRAS-driven cancers. The Initiative is based at Frederick National Laboratory for Cancer Research in Frederick, MD, and involves multiple collaborations with the RAS research community in academia and industry with the shared goal of developing RAS therapies.

INTRODUCTION

Forty years have passed since *RAS* mutations were identified in human tumor DNA. A single amino acid change converts a normal protein to a powerful oncogene: this seemed, in 1982, to present a golden opportunity to develop therapies based on the simplest imaginable difference, but after 30 years of failed efforts, Ras proteins were classified as undruggable. However, the importance of K-Ras as a cancer driver had been clearly demonstrated, and the urgency to develop therapies targeting K-Ras was underscored by failure to improve outcomes of pancreatic cancer, a KRAS-driven disease, and by the exclusion of *KRAS*-mutant lung adenocarcinoma or colorectal carcinomas from therapies targeting EGFR, leaving patients with KRAS cancers with no satisfactory options. Signs of renewed efforts to target K-Ras emerged in 2012: drug discovery efforts at Genentech and Vanderbilt, using nuclear magnetic resonance (NMR)–based fragment screens, identified molecules that bind to K-Ras directly (1, 2).

A combination of an urgent clinical need and the recognition of the power of new technical advances in drug development, such as those employed by Genentech and the Fesik group at Vanderbilt, prompted Dr. Harold Varmus, the director of the NCI, and colleagues to believe that there was an opportunity to attack recalcitrant targets such as Ras with new focus. There were major knowledge gaps to be filled that could enable new drug discovery efforts. For example, there were no structures of any codon 12 mutant of Ras protein in the Protein Database, despite the recognition that these proteins were prime drug targets. Other knowledge gaps included a poor understanding of how Ras proteins interact with each other in the membrane, how they activate their effectors, and which effectors are most important. Shedding light on any one of these questions could help advance strategies for targeting *RAS*-driven cancers.

To address these needs and opportunities, Dr. Varmus initiated a discussion within the NCI and extramural Cancer Research Community to use the unique capabilities of NCI's Federally Funded Research and Development Center at Frederick, MD, known as the Frederick National Laboratory for Cancer Research (FNLCR), to address challenges such as that of RAS cancers. Dr. Varmus called a meeting of cancer experts from across the nation where a consensus to establish a focused effort on K-Ras was confirmed. Through discussions with Dr. David Heimbrook, the laboratory director of FNLCR, Dr. Varmus challenged FNLCR to propose a pivot of effort that leveraged existing funds and resources to form the RAS Initiative.

Dr. Frank McCormick was invited to serve as scientific director of the RAS Initiative, and he worked with FNLCR leadership to develop goals, evaluate resources, and assemble a team that formed an experimental and drug discovery core that would collaborate closely with the academic and biopharmaceutical industry in a hub-and-spoke model. These plans were approved in 2013 by a joint meeting of the Board of Scientific Advisors and the National Cancer Advisory Board (https://deainfo.nci.nih.gov/advisory/joint/0613/index.htm) and by the Frederick National Laboratory Advisory Committee (https://deainfo.nci.nih.gov/advisory/fac/archive/0913/index.htm), which was established to vet and oversee work at the National Lab. An *ad hoc* RAS Initiative advisory group was also established, initially chaired by Dr. Levi Garraway and now chaired by Dr. David Tuveson.

ENABLING THE RAS COMMUNITY

A key objective of the RAS Initiative was to nucleate and enable an extended RAS research community through a hub-and-spoke model. Early efforts to enable the research community included generating a standard set of expression vectors for the more than 200 genes in the Ras pathway. This pathway was defined with iterative input from the RAS research community, resulting in the Ras pathway 2.0 (Fig. 1) cDNAs encoding all the proteins in Ras Pathway 2.0 (https://www.cancer.gov/research/key-initiatives/ras/ras-central/blog/2015/ras-pathway-v2) were checked and obtained, many of which had contained sequencing errors or encoded the wrong splice variant. Quality controlled clones were made available through Addgene to facilitate efficient distribution to the RAS community. Protocols for producing fully processed KRAS4B protein to the RAS community were developed, as well as an isogenic panel of single-isoform RAS-driven mouse embryo fibroblasts to help the community determine drug specificity. A *RAS Interactome* was created

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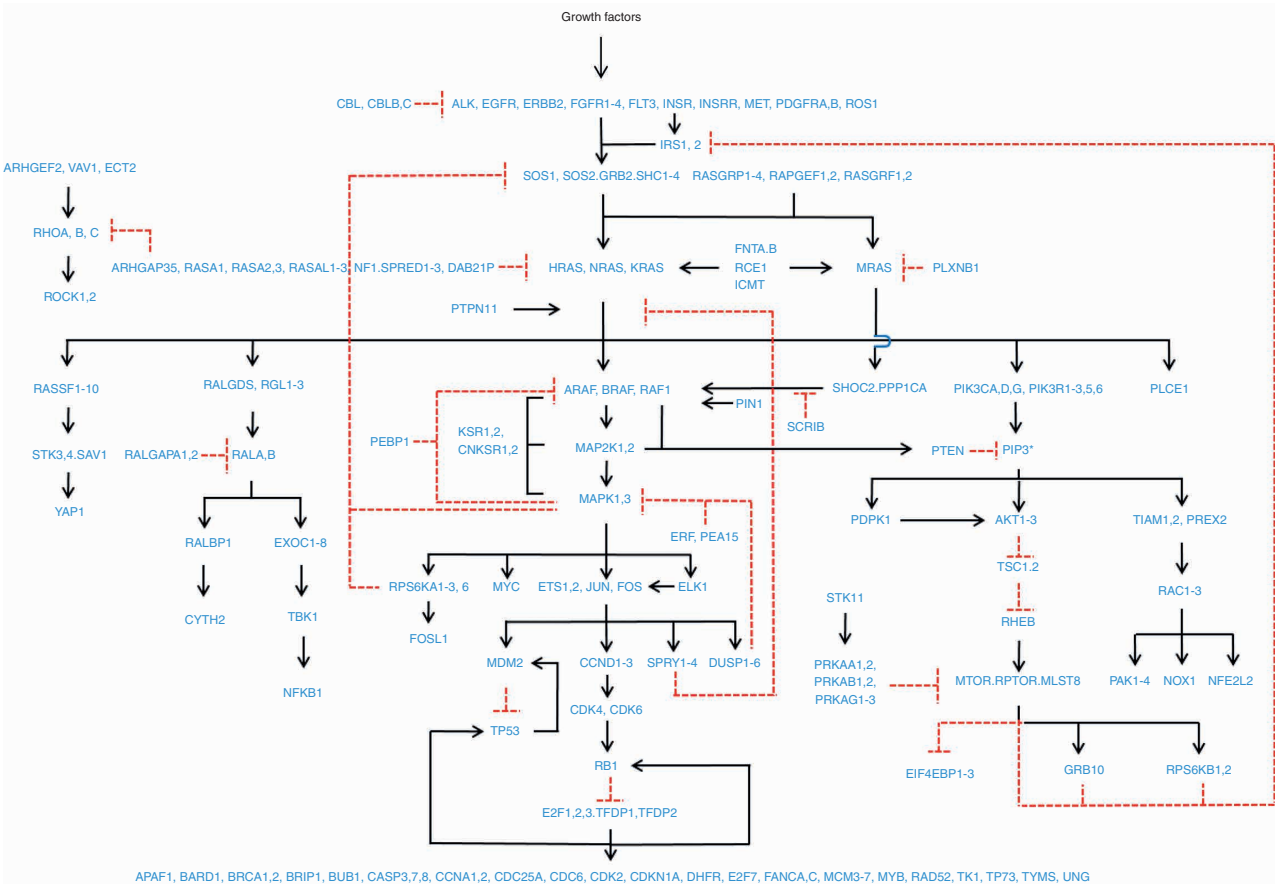

Figure 1. The Ras Pathway 2.0.

through cancer.gov/RAS and the interactive online platform RasLab to distribute information on work being done at the Frederick National Lab, RAS Biology blogs, and exchange of experimental information, ideas, and scientific discourse/debate. There are currently over 1,100 members of this discussion group, and 400 discussions have been logged to the site to date, providing a unique opportunity for the Ras community to interact and help each other in real time. Three major international symposia at Frederick, MD have also helped bring the community together, and another is planned to discuss the latest discoveries.

STRUCTURAL BIOLOGY

Because a major objective of the RAS Initiative is to generate drug discovery enabling structural insights into K-Ras, its oncogenic alleles, and K-Ras in complex with GEFs (Guanine Nucleotide Exchange Factors), GAPs (GTPase Activating Proteins), and effectors, we complemented our state-of-the art protein expression facility by focusing on structural biology efforts. In close collaboration with the protein production team, the structures of several oncogenic K-Ras-mutant proteins in their active and inactive states were solved for the first time, as well as fully processed K-Ras complexed with PDEδ—the first structure of any RAS protein in its full length, fully processed state (3); the K-Ras/neurofibromin/Spred1 complex (4); the K-Ras/Raf-1 RBD/CRD complex (5); Raf/Sin1 (6); and several other complexes, using conventional X-ray crystallography as well as cryo-electron microscopy. The structural biology group plays a central role in our drug discovery efforts, in close collaboration with the NMR, biophysics, and biochemistry teams.

DRUG DISCOVERY

In 2013, Shokat and colleagues (7) published their landmark article describing molecules that bind covalently to K-Ras^{G12C} and lock it into its inactive state. This triggered a stampede of G12C inhibitors, all binding in the same pocket and with similar chemistries. Covalent binding solved the problem of finding small molecules that interact with Ras proteins with high affinity, considering its lack of suitable pockets. The RAS Initiative also pursued covalent approaches, building off work performed earlier by the McCormick laboratory at UCSF, in which we screened for compounds that bound covalently to C185, the site of prenylation of K-Ras 4B, using the same tethering library at UCSF used by Shokat and colleagues. Compounds that bound the G-domain of K-Ras and bound covalently to C185 were identified and developed (Maciag, A. and colleagues, unpublished data)

but have not been advanced further because, unexpectedly, these compounds only react with the K-Ras 4B isoform, not the K-Ras 4A isoform, and this is likely to limit their efficacy. However, in the course of these experiments, we also identified compounds that react covalently and, unexpectedly, with residue H95 on K-Ras. This residue is unique to K-Ras (both 4A and 4B isoforms). These insights led us to propose a "pan-K-Ras" strategy in which small molecules inactivate K-Ras specifically through engagement with H95 and were the genesis of a major drug discovery effort in collaboration with the biotechnology company TheRas and with the computational biology group at Lawrence Livermore National Labs. This collaboration now includes compounds that target the GTP-bound form of K-Ras^{G12C} and other specific alleles and compounds that prevent Ras protein binding to PI 3′ kinase-alpha. We anticipate that clinical development compounds from these programs will be selected in 2022 and will enter clinical testing soon thereafter.

Another important drug discovery campaign was launched in collaboration with Sanofi. This effort is based on structural insights from the structure of the K-Ras/Raf-1 RBD/CRD complex (5). This analysis identified a binding surface between K-Ras and the CRD region of Raf-1 kinase, which is critical for Raf-1 kinase activation, although not for Raf-1 binding. This binding interface is relatively low affinity and differs between Raf isoforms, making it an attractive target for therapeutic intervention. This example also validates one of our original hypotheses, that solving structures of oncogenic forms of Ras bound to their effectors might reveal new opportunities for intervention. We have also deployed cell-based screens to identify compounds that prevent Ras binding to Raf, in collaboration with Eli Lilly.

As our drug discovery projects advanced, it became necessary to establish an in-house synthetic/medicinal chemistry program to establish novel chemical libraries and methods for tethering-based drug discovery, which led to a new 2,000 compound cysteine tethering library that is being screened against a protein library in which all surface-exposed K-Ras residues are mutated to cysteine to identify small molecules that interact with unrealized or cryptic pockets.

Ras IN MEMBRANES

The topic of Ras clustering and oncogenic signaling in the context of the plasma membrane has long been of interest to the field but difficult to study in part due to the lack of reagents and tractable experimental systems. The RAS Initiative made a significant contribution in this area by working out the production of fully processed (farnesylated and carboxymethylated) K-Ras 4B (8). This reagent has been used to look at K-Ras 4B when bound to membrane mimetics using biophysical, biochemical, and structure-based approaches within the Initiative and across the field.

As these studies were being conducted in the Initiative, the National Strategic Computing Initiative (July 2015) resulted in the NCI and Department of Energy forming a partnership to develop high-performance computing applications to address difficult biomedical research challenges (Joint Design of Advanced Computing Solutions for Cancer). The RAS Initiative established a collaboration with teams from Oak Ridge, Argonne, Los Alamos, and Livermore National Laboratories to model computationally the behavior of Ras molecules on complex, eight-lipid bilayers and the process of RAF kinase activation through iterative experiment-computation cycles. This interdisciplinary approach resulted in a novel artificial intelligence–aided multiscale simulation capability that models membrane biology at experimentally accessible time scales and provides new insights on Ras–Ras and Ras–lipid interaction during the formation of nanoclusters and the initiation of signaling.

THE FUTURE

In 2022, we expect to embark on our first clinical trials, most likely with our G12C.GTP inhibitor in collaboration with TheRas. In the following years, we hope to develop compounds that target different *RAS* alleles, including the major driver of pancreatic cancer, G12D, and compounds that prevent activation of Raf and PI 3′ kinase-alpha by oncogenic RAS, as well as other approaches. Drugs derived from the RAS Initiative, as well as from major development efforts in biotechnology companies such as Mirati, Quanta, Revolution Medicines, and Frontier Medicines, all of which have declared their plans to develop K-Ras inhibitors, as well as major pharmaceutical companies such as Amgen, Genentech, Sanofi, Eli Lilly, Novartis, and Boehringer Ingelheim, will occupy a central position in oncology drug development for the decade that follows the 40th anniversary of the discovery of *HRAS*^{G12V} in human DNA, exploring multiple combinations and modalities and dealing with the inevitable problems of drug resistance.

Progress in drug discovery will be greatly accelerated by advances in computational and structural biology, which could bring other members of the RAS family to the party, including N-Ras, Rac, and Rho. These proteins are remarkably similar to Ras in their G-domains and could yield to direct targeting based on lessons learned from targeting Ras.

From a more fundamental perspective, we hope to see real progress in understanding precisely how Ras proteins function in the membrane and how signaling complexes are assembled and regulated. Further downstream, we may learn precisely how persistent signaling from oncogenic RAS proteins causes cancer and will develop more therapies based on these insights. At that point in time, probably when we celebrate 50 years of Ras, with drugs approved for treating most RAS cancers effectively, we can declare that the original mission has been accomplished.

Authors' Disclosures

D.V. Nissley reports that the RAS Initiative has established cCRADAs with Eli Lilly, Evotec, Progenra, Sanofi, and TheRas to jointly discover and develop inhibitors of KRAS signaling. The RAS Initiative receives funds from TheRas for collaborative work. D.V. Nissley receives no personal funding or compensation as a result of these cCRADAs and is currently not an inventor of any associated intellectual property. F. McCormick reports personal fees from Leidos Biomedical during the conduct of the study; personal fees from BridgeBio, Pfizer, Amgen, PMV, Quanta, Frontier Medicines, Kura Oncology, and Ideaya outside the submitted work; and a patent for 25. US Patent No. 10,857,140 Kras Modulators-12/8/2020 issued, licensed, and with royalties paid from BBIO.

Disclaimer

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