

RAS Program

at the Frederick National Laboratory

The Frederick National Laboratory for Cancer Research (FNLCR) is an applied research laboratory and a unique national resource as the only U.S. national laboratory devoted exclusively to biomedical research. The mission of FNLCR is to accelerate treatment for cancer and AIDS patients through innovative basic research, development of new technologies, and the translation of basic discoveries into the clinic.

Leveraging Cross-disciplinary Capabilities to Enable Scientific Discovery

KRAS is known to be a driver in pancreatic, colorectal, and lung cancer. To date, chemical approaches to directly inhibit KRAS have been unsuccessful, and further understanding of KRAS signaling and regulation may translate into improved treatments for RAS-driven cancers.

With the unanimous endorsement from both the National Cancer Advisory Board and the NCI Board of Scientific Advisors, FNLCR has launched the RAS Program to identify and attack significant unmet scientific challenges posed by KRAS-driven cancers. Frank McCormick, Ph.D., renowned expert in the field of RAS biology, has recently joined the FNLCR team as a consultant to assist in the leadership of the program, along with the director of FNLCR, David Heimbrook, Ph.D. FNLCR scientists will carry out a number of intra-linked projects that employ the extensive resources resident at the national laboratory in protein chemistry and biophysics, imaging, and genetics and genomics. FNLCR will serve as the research hub that connects to research collaborators nationwide. Through these collaborative activities, the goal of the program is to elucidate the underlying molecular mechanism of KRAS in cellular transformation and translate those results into more effective treatments for KRAS-driven cancers.

RAS Program Projects

Validation of KRAS as a therapeutic target

To validate KRAS as a target for therapeutic intervention, determinations will be made of the profile of KRAS-driven tumors that respond to ablation of mutant KRAS and of the pathways acquired in the course of resistance to KRAS ablation.

FNLCR will draw on its capabilities in cell line development and vector engineering, as well as on its bioinformatics expertise, to introduce relevant KRAS mutations into selected cell lines using a combination of allelic replacement and/or knockdown approaches. Complementing these studies are cellular assays to examine

transformation states such as anchorage-independent growth and migration/invasion, along with proteomic and gene expression profiling. Individual project components will include:

- Characterization of mutations in primary and metastatic tumor cell lines
- Cell-based assays to assess the effect of KRAS modulation on phenotype

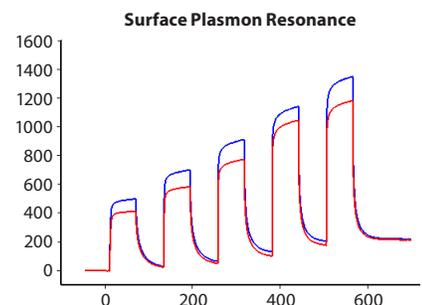
The research tools and assays developed in this project will be critical for the support of the remaining RAS projects.

Identification of novel KRAS structures and interfaces

Using biophysical approaches, further analysis of KRAS, as well as KRAS complexed with binding partners, will be performed to identify new structures and protein interaction interfaces. Further understanding of structure and complex functionality will support in vitro assay development for the analysis of potential therapeutics. Individual project components will include:

- Development of protein and activity analysis of KRAS
- Structural analysis of relevant KRAS alleles with regulators and effectors
- Definition of KRAS complexes isolated from cells
- Biophysical analysis of KRAS binding to calmodulin and other partner proteins

The project will utilize resident expertise in protein production, biophysical protein analyses, and mass spectrometry-based protein complex identification. Additionally, the project will develop in-house pilot protein crystallization capabilities.

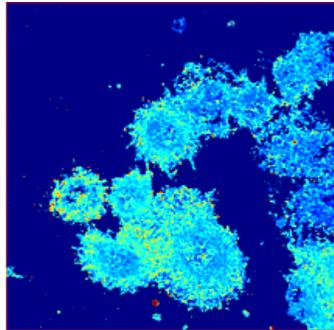


Genotype-independent KRAS compound identification

In order to identify compounds that specifically inactivate KRAS independent of genotype, FNLCR scientists will develop assays that monitor membrane localization, calmodulin binding, and post-translational modification and processing of KRAS.

Additionally, this project will examine the significance of other types of modification to KRAS proteins, such as ubiquitination and acetylation, to determine whether these modifications offer new opportunities for therapeutic intervention.

FRET Analysis of RAS

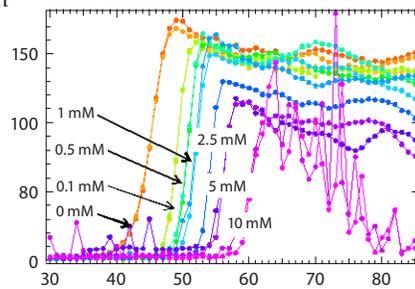


Disruption of KRAS complexes

The objective of this project is to further characterize KRAS complexes in cells and elucidate the mechanisms of KRAS multimerization. Understanding the regulation of KRAS complex formation and interaction with the membrane will allow the development of assays to screen for disruption and mis-localization of KRAS complexes.

FNLCR's expertise and infrastructure in high-content imaging are being used to verify KRAS protein complexes in cells and to probe the nature of KRAS dimerization. These techniques will validate the biochemical analysis of KRAS effector complexes. Disruption of signaling complexes consisting of dimers and/or higher-order structures presents an attractive new opportunity for drug discovery. High-content imaging screens will be used to facilitate the screening of chemical libraries.

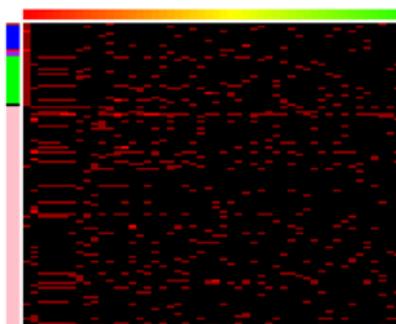
Static Light Scattering



Mapping surfaces of KRAS transformed cells

Deep molecular description of the surfaces of KRAS cancer cells is being carried out to gain further understanding of differences between cells expressing wild-type or mutant KRAS alleles. This includes characterizing the surface protein content under physiological conditions, under clinically relevant conditions such as hypoxia, and during exposure to therapeutic protocols (e.g. chemotherapy, radiation, etc.). Using the cell biology and cell genetics capabilities at FNLCR, isogenic cell types that differ only in KRAS genotype are being generated for

RAS Co-mutation Analysis



characterization. In conjunction with cell surface labeling and cell fractionation, mass spectrometry will be used to determine differences in membrane proteins between paired cell types. Also being explored are alternative approaches to probing cellular surface protein content that include phage display, direct biochemical methods, and informatics-based analyses of databases. These approaches will be integrated to develop a comprehensive map of surface proteins on specific KRAS-driven cancers.

Next-generation synthetic lethal screens

Identification and validation of KRAS targets using next-generation synthetic lethal screens is a goal of the RAS Program. Still in conceptual stages, assessment is taking place for the possibility of performing synthetic lethal screens in vivo and/or in vitro using RNAi or CRISPR technologies. Through an ongoing series of workshops and interactions with leaders in the field of synthetic lethal screening, the best approaches for carrying out KRAS-specific screens will be defined. FNLCR is considered the coordination center for facilitating collaborative research in this area.

Production and validation of reference reagents

One of the important outcomes of the RAS Program, in addition to supporting internal research, is the generation of qualified and standardized reagents that will be made available for the extramural RAS research community. FNLCR will become a national resource center for these reference-quality RAS research materials. Reagents will include DNA clones, cell lines, viruses, antibodies, and proteins. The generation of these reagents will be directed by both the research needs of the national laboratory as well as the consensus of the RAS research community, to enable scientific discovery at both FNLCR and in the wider scientific community.

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