

Report from the RAS *ad hoc* working
group on the FNLAC RAS Initiative

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Virtual meeting of the Frederick National Laboratory
Advisory Committee

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Mission of the FNLAC RAS Working Group

To advise strategic, technical, and scientific aspects of the NCI RAS Initiative

To present findings and recommendations to the Frederick National Laboratory Advisory Committee (FNLAC)

Specific goals:

- (1) provision of feedback and suggestions to Dr. Frank McCormick and his team at FNLCR
- (2) regular, candid assessments of aspects that are working well and areas where improvements or pivots might be needed
- (3) ensuring optimal connectivity between the FNLCR RAS initiative and the extramural community

Seven WG meetings held between July, 2014 and August, 2016

Biophysical Characterization of KRAS

Highlights:

- Purified fully processed (farnesylated, methylated) KRAS protein and characterized GTPase activity
- Characterized binding to RAF on nanodiscs (published in *Nature Scientific Reports*)
 - RAS is away from the bilayer in potentially 2 RAF-binding conformations
- Ongoing studies are pursuing cryo-EM, surface plasma resonance
- Collaboration in progress with Department of Energy to use supercomputing to build models of membrane RAS/RAF interactions

KRAS Structural Biology

Highlights:

- Crystal structures of wild-type KRAS and 6 oncogenic mutants have been solved
 - These revealed how oncogenic mutations affect the conformation of regions that interact with regulatory and effector proteins and perturb GTP hydrolysis
- Crystal structures of Q61 mutants complexed with the GAP protein, RASA1, were also solved
- Full length, fully-processed (farnesylated, methylated) KRAS complexed with the chaperone PDE δ was solved
 - These structures reveal the hypervariable (C-terminal region) of KRAS for the first time and sequences important for interaction with PDE δ

Assay Development and Screening

Highlights:

- Cell-based assays were developed to monitor RAS dimerization, membrane localization, effector activation, and cell proliferation
 - Examples include NanoLuc[®] to assess KRAS-RAF interaction, isogenic MEF cell lines that express wt HRAS or mutant KRAS
- *In vitro* assays have been developed to measure RAS GTPase activity, lipid interactions, and effector interactions
- Multiple pharma and biotech partners for small molecule screens

Interactions with the Scientific Community

Highlights:

- RAS reference reagents: RAS pathway clones (180 genes) and reagents for making fully-processed KRAS requested by researchers from 23 countries
- Formal collaborations with Pancreatic Cancer Action Network to fund trainees, DARPA's big mechanism project, DOE, RAS Synthetic Lethal grantees, and 53 individual scientists and companies
- Promoting discourse and disseminating information by its website and blogs (cancer.gov/ras) and an interactive website (basecamp) where 650 scientists discuss RAS research challenges and ideas
- Hosted 7 targeted workshops and a RAS Initiative Symposium (attended by 550 researchers)

Conclusions

The RAS Initiative has had expert direction from Dr. McCormick

World-class set of project objectives defined up-front

Talented staff

Has achieved considerable success in each of its projects

- new insights into RAS biology

- developed reagents and assay platforms available to academics and industry

Has catalyzed interest in doing RAS research; and increased awareness in the importance of RAS biology

Well positioned to serve as robust platforms for current and future collaborations and to accelerate the development of RAS-directed therapies

Recommendations

- The burgeoning industry collaborations that involve this RAS initiative should be used to identify lessons learned that guide development of a generalizable framework for how such activities might be extended going forward. Examples might include systematic target validation approaches, establishing avenues capable of further lead compound development, and new types of service models to engage the broader community.
- Continuing development of large-scale approaches to both identify and validate new compounds that selectively inhibit wild-type or mutant RAS. In particular, future efforts could determine how the new (and emerging) RAS structures can inform drug screens. Of particular interest might be the expansion of in silico screens given that the DOE has a 1 billion compound library.
- Further augmentation of the biochemical advances should be prioritized: one goal herein might be to develop an in vitro system in which RAS successfully functions in the plasma membrane to activate effectors.
- Consider efforts to resolve RAS-effector complexes using cryo-EM.