FNLCR RAS Working Group Meeting--Summary

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RAS Working Group: Mission

The purpose of this working group is (1) to provide the highest quality oversight to the technical aspects of the RAS Program; and (2) provide its findings and recommendations to the NCI Frederick Advisory Committee (NFAC) and the *ad hoc* RAS subcommittee.

This will include assessments of:

- Scientific goals, directions, priorities, and timelines of RAS research projects at FNLCR.
- Engagement of the extramural community and industry by the NCI RAS Program in sharing ideas, reagents, and data.

Objectives for the initial RAS working group meeting (July, 2014)

- Review major initiatives linked to all major RAS project components
- Provide feedback and suggestions to Dr. Frank McCormick and his team at FNLCR
- Make a candid assessment of where things are working well and where optimization might be needed
- Assess initial efforts at connectivity with the extramural community

RAS Program Overview-1 (McCormick)

- NCI resources have been transitioned so that approximately 50 people are now working on the RAS Program at FNLCR.
  - The RAS Program receives $10 million annually from NCI-directed re-prioritization
  - No new money
- "Hub and spoke" model: FNLCR (hub) interacts with extramural NCI-supported academic laboratories, biotech companies, pharmaceutical companies, and contract research organizations
  - E.g., U01 mechanism announced in August ("next-gen" synthetic lethal screens beyond 2-D culture)
  - Postdoctoral fellows program at FNLCR

RAS Program Overview-2 (McCormick)

- ***Project 1:***
  - determine which RAS effectors are engaged by each of the mutant proteins
  - solve structures of mutant proteins in complexes with relevant effectors
  - Characterize RAS post-translational processing
- ***Project 2:*** develop cell-based and phenotypic assays to identify KRAS-selective compounds
- ***Project 3:***
  - imaging KRAS complexes in cells
  - developing screens for compounds that disrupt complexes or signaling effectors
- ***Project 4:*** map the surface of KRAS-mutant cancer cells to identify "antigens" that could be targeted by immunotherapy or nanoparticles.
- ***Project 5:*** develop next-generation KRAS synthetic lethal screens; implement various mechanisms to interface with the extramural community

RAS Structural Biology (Project 1)
Andy Stephen

- The goal of this component is to create useful structures of KRAS with its various interacting partners:
  - (1) generate high-quality protein reagents for biochemical and biophysical analysis and support assay development
  - (2) determine structures of KRAS oncogenic mutants and complexes with interacting partners (GAP and calmodulin)
  - (3) comprehensively characterize biophysical parameters of key KRAS interactions to identify optimal targets for drug design
  - (4) develop methods for producing processed KRAS at high yield and quality for biophysics and structural biology
  - (5) collaborate with the external RAS research community on structural biology efforts.

Challenges in RAS Structural Biology

- Crystallization conditions and crystal packing affects the switch I conformation
- There is currently no structural biologist on the RAS FNLCR staff, so the group has been working with extramural collaborators
  - Crystallization and structure determination is currently being done with a CRO and extramural investigators
- GAP structures complexed with RAS would be useful but are difficult to crystalize
- The specific effectors bound by mutant RAS in cancer cells remain incompletely understood

RAS Structural Biology: Working Group Suggestions/Recommendations

- Crystal structures of mutant RAS oncoproteins could represent an obvious low-hanging fruit for early characterization and dissemination to the community.
- May be worth considering contacting pharmaceutical companies that have previously done or attempted RAS structural biology for possible collaboration
- Patient-derived pancreatic cancer cells can now be cultured as organoids and might provide enough material for pull-down experiments
- Study the structure of multiple GAPs complexed to RAS to learn the “rules” of such interactions and support *in silico* screens for compounds that stabilize RAS-GAP
- Establishing whether intrinsic GTPase activity is crucial or if it is only GAP stimulated activity that is important.
- Establish clear project milestones (including go/no-go decisions)

RAS Processing (Project 1) (Dom Esposito)

- Prenylation of RAS proteins is required for their activity.
- Currently, prenylated RAS proteins are being produced using baculovirus technology, which implements eukaryotic post-translational modifications.
- To conduct a structural and biophysical analysis of the fully processed KRAS protein, liposomes will be prepared.
  - This should enable crystallography, nuclear magnetic resonance, electron microscopy, and characterization of biochemical and biophysical parameters with and without effectors.

RAS Processing: Challenges

- Insect cells are not effective at farnesylation (thus, human FTI was engineered)
- A methyltransferase also needed to be co-infected
- A substantial amount of unprocessed KRAS remains after these manipulations
- Next step is to integrate these modifying enzymes into the genome to prevent incomplete infection, the likely cause of unprocessed protein.

RAS Processing: Working Group Suggestions/Recommendations

- Large-scale production of RAS proteins is an example of a concrete deliverable that would be of considerable benefit to the community.
- These resources—particularly in membrane based liposomes—would like be in high demand from academic and pharmaceutical sectors
- Priorities for FNLCR will include:
  - development of robust production quality standards
  - mechanisms for sharing these resources with the research community

KRAS-Effector Interactions and Cell-based Assays (Project 1)

(Andrew Stephen and Matt Holderfield)

- The goal of studying the interactions between KRAS and its effectors is to develop drugs that could disrupt these interactions.
- A key need in this regard is the development of RAS effector binding assays and read-outs of RAS dimerization.
- Three different assays are currently being developed
  - the cell-based AlphaScreen
  - bimolecular fluorescence complementation assay (constitutive mCherry for cell proliferation)
  - bioluminescence resonance energy transfer assay
- Many of these would be secondary rather than primary drug screening assays

KRAS-Effector Interactions and Cell-based Assays: Workgroup Suggestions

- Concentrations of KRAS and C-RAF in cells are about 100 fold less than in the current assay conditions
  - (also, A- and B-RAF should not be ignored)
- Retain compound “hits” that stabilize (as well as destabilize) RAS-effector interactions--these too could be useful for drug discovery
- Don’t allow primary screening assays to become too complicated
- Gain access to as many screening libraries as possible (beyond NCATS)

Phenotypic Assays (Project 2)
(Turbyville, Bagni, Soppett)

- Development of RAS-null mouse embryonic fibroblasts, which do not proliferate: rescue proliferation with various RAS isoforms
- Develop RAS isoform-dependent proliferation screening strategies
- Goals for FY2015 are to complete cell-line testing and develop a high-throughput screen for human epithelial cell lines using an engineered endogenous *KRAS* locus

Phenotypic Assays: Workgroup Recommendations

- Several potential source of false positive and false negative results were noted with this assay approach
- There are also technical challenges in the development of competitive growth assays
- Develop controls to assess these possible pitfalls as the project evolves

Mapping the Surface of KRAS Cancer Cells (Project 4) (Gordon Whiteley)

- The goal here is to identify cell surface proteins that are differentially associated with the KRAS phenotype
- The team will use mass-spectrometry based interrogation of wild-type and KRAS-driven cell lines, bioinformatics data mining, and cross-validation with other approaches
- In preliminary studies, the team identified 666 cell surface proteins unique to MCF10A KRAS cells (8 of these proteins were identified by two orthogonal approaches)

Mapping the Surface of KRAS Cancer Cells: Workgroup Recommendations

- The efforts were felt to be interesting albeit preliminary:
- It was not clear how to incorporate positive and negative controls for KRAS-dependent cell membrane expression differences.
- Comparing a mutant KRAS-expressing, lung tumor line to a B cell line (wt KRAS) from the same patient might be confounded by lineage or cell line specific effects.
- The use of mouse cells for validation studies could miss human-specific KRAS effects
- Multiple independent KRAS wt and mutant cell lines (ideally from a human cancer cell context) are needed to identify cell surface proteins that segregate with KRAS
- Collaboration with leading membrane proteomics labs would be helpful

RAS Signaling Analysis (Project 3)

- RAS activates different effector arms. The goal of this component is to generate broad and deep "perturbagen" data to construct better models of RAS signaling and possible downstream targets.
- In a series of KRAS-mutant cancer cell line models, one goal is to knock-down KRAS and/or downstream signaling nodes and measure various hallmarks of cancer.
  - In preliminary studies, some effects of KRAS knockdown are unexpected
- Identify genomic determinants of variability in the phenotype of KRAS-mutant cell lines and evaluate the distribution of these genomic events across public data sets

RAS Signaling: Workgroup Recommendations

- The fluorescence (eGFP) based visualization of node knockdowns may need deeper characterization under well controlled conditions so that assay variability (and determinants thereof) can be better understood.
- Suggestions were also made as to how best to represent the signaling data.
- Since eGFP and RAS signaling can both induce ROS, which may confound data interpretation
- Comparison of results from UCSF and FNLCR labs will also be important to ensure assay robustness

➢ While exciting, the signaling project data is preliminary, moreover the effort is ambitious and possibly lengthy. Thus, clear metrics/deliverables are needed to track and communicate progress.

RAS Bioinformatics

- This aspect of the RAS program will:
  - build and maintain a data repository to house RAS project data
  - provide a mechanism to integrate this data with public data sources
  - provide direct analysis support to specific projects
  - perform data mining to answer questions about the function of KRAS-mutant alleles in different biological contexts
- Initial study of ratio of KRAS 4A and 4B isoforms in different settings

➢ It was recommended that RAS bioinformatics personnel connect with TCGA investigators or other extramural computational biologists with familiarity in mining this resource.

RASCentral and Reference Reagents-1 (Hartley and Esposito)

- The goal of RASCentral is to facilitate communications, share results, and encourage collaborations between the RAS intramural and extramural communities.
  - seminars at FNLCR,
  - outside collaborations,
  - intramural collaborations
  - RAS workshops
- http://www.cancer.gov/ras
- It was suggested that organizing a mini-symposium on RAS at a major meeting, such as the meeting of the American Association for Cancer Research (AACR), could help communications regarding the FNLCR RAS project

RASCentral and Reference Reagents-2 (Hartley and Esposito)

- An important role of the FNLCR RAS program is to generate high-quality reference reagents for distribution to the RAS external community. (This activity is consistent with a role that FNLCR has played for other research.)
- DNA vectors, cell lines, viruses, proteins, and antibodies.
- Need to determine how to vet reagent requests and distribute resources--all who request such reagents should have a facile mechanism to get them
- Guidance from the community will be helpful to determine where demands will be greatest

RAS Program and Pharma Partnerships (Heimbrook)

- A face-to-face meeting between FNLCR and pharmaceutical companies was held at AACR in San Diego in April 2014
  - Follow-up planning workshop in August
- Exploring a FNLCR-pharma consortium focused in the precompetitive space
- RAS program postdoctoral fellows could provide the “glue” in such collaborations

RAS Program and Pharma Partnerships: Challenges

- Government regulations and boundaries may present major obstacles to pharmaceutical collaborations
- Such constraints (and strategies to circumvent them) should be clarified early
- An NCI consortium might collaboratively fund projects with pharma for outside partners such as academics or early stage clinical trials
- Awareness of the program and its collaborative goals needs to be expanded (many still do not know much about this FNLCR effort)

Additional Working Group Recommendations and Next Steps-1

- McCormick: immediate priorities include:
  - launching an FNLCR-industry consortium
  - implementing a postdoctoral fellow program at FNLCR
  - developing a community through RASCentral
  - hosting RAS scientific conferences both at Frederick and appended to existing meetings
  - Workshop on immunotherapy in RAS cancers

Additional Working Group Recommendations and Next Steps-2

- The crystal structures of the major cancer-associated RAS mutants could be an important early publication
- A “RAS-centric” TCGA analysis could represent a useful computational study
- Immediate launch of the RAS postdoctoral fellows program should be pursued
- KRAS based proteomics studies could be valuable to the community but intensive efforts are needed to optimize design of this component
- Characterizing tumor-infiltrating lymphocytes present in RAS tumors (and how to activate them to kill the tumor cells) could be an important immunotherapy interface
  - A workshop on this general topic could be important

Additional Working Group Recommendations and Next Steps-3

- Bringing additional intellectual leadership to FNLCR in key areas may be needed (e.g., proteomics, structural modeling, etc.) One option would be to recruit experts using a part-time model such as that adopted by Dr. McCormick.
- Clear goals, deliverables, and near-term objectives should be set for those projects that are most preliminary at present.
- Future working group meeting plans: conference call once per quarter and a yearly meeting in Washington, D.C.
  - Conference call to be set up for the fall
  - Meet during AACR Annual meeting in April, 2015