Ras Initiative

Frank McCormick
Structural Biology and Biochemistry
The structural and biochemical properties of KRAS and its most prevalent mutants will be characterized to look for ways to modulate their activity.

RAS Assays
New assays for RAS activity may be useful tools to screen for RAS pathway inhibitors.

Biology of Mutant KRAS Cell Lines
Commonalities in dozens of cell lines derived from human cancers that have mutant KRAS genes could reveal insights into selective vulnerabilities for treatment.

Pathways Analysis
Surprising failures of new cancer treatments have made it clear that we do not know enough about how molecules in RAS signaling pathways interact with each other.

Cell Surface Analysis
Identifying cell surface features specific to mutant KRAS cancers could give us unique opportunities to develop treatments that target the cell surface.

RAS Reference Reagents
An important priority of the RAS Initiative is to distribute highly validated materials and methods to the world-wide community of RAS researchers.
Implementing the RAS Program
Hub, Spoke, and RAS Community model

FNLCR – The Hub

Intramural Labs

Extramural NCI-Supported Labs

Biotechs

Pharma

Advocacy
Parameters affecting normal Ras activity

Growth Factors

GEF-mediated GDP dissociation

GAP-mediated GTP hydrolysis

GDP

GTP

RalGDS

RAFs

PIK3CA
Parameters affecting oncogenic Ras activity

Growth Factors

GEF-mediated GDP dissociation

GAP-mediated GTP hydrolysis

GDP  \[\xrightarrow{\text{GAP-mediated}}\text{GTP}\]  GTP

RalGDS  RAFTs  PIK3CA
Distinct biological and clinical properties of KRAS alleles

-KRAS G12V, G12C: worse clinical outcome than G12D (lung cancer) (Al-Mulla et al; Andreyev et al; Vega et al; Keohavong et al)

-KRAS G13D: respond to Cetuximab (colorectal cancer) (de Roock et al, 2010)
Isogenic cell lines from RAS-less MEFs

HRAS^-/- NRAS^-/- KRAS^lox/lox MEFs

Drosten M, Dhawahir A, Sum EY, Urosevic J, Lechuga CG, Esteban LM, Castellano E, Guerra C, Santos E, Barbacid M. EMBO J. 2010

HRAS

\[ \downarrow \]

eGFP-WT

eGFP-G12V

PAmCherry-WT

WT

G12V

NRAS

\[ \downarrow \]

eGFP-WT

WT

G12V

KRAS4A

\[ \downarrow \]

eGFP-WT

WT

eGFP-mutant

G12V

G12D

G12C

G13D

Q61L

Q61R

KRAS4B

\[ \downarrow \]

eGFP-WT

WT

eGFP-mutant

G12V

G12D

G12C

G13D

Q61L

Q61R
HRAS\textsuperscript{\textminus/} NRAS\textsuperscript{\textminus/} KRAS\textsuperscript{lox/lox} MEFs

Untreated MEFs

G1 arrest (day 19\textsuperscript{*})

+4-OHT

Re-enter cell cycle

Drosten M, Dhawahir A, Sum EY, Urosevic J, Lechuga CG, Esteban LM, Castellano E, Guerra C, Santos E, Barbacid M. EMBO J. 2010
HRAS^{WT} vs KRAS^{G12D} MEF proliferation screen

- Compound library was provided by NCATS (National Center for the Advancement of Translational Sciences)
- The library is enriched for “tool” compounds, but also contains FDA approved drugs

Kanika Sharma (FNLCR), Kyle Brimacombe (NCATS)
Receptor Tyrosine Kinase (RTK) inhibitors

![Graph showing the relationship between KRAS G12D IC50 and HRAS wild-type IC50 with RTK inhibitors highlighted.]

Kanika Sharma (FNLCR), Kyle Brimacombe (NCATS)
Full-length KRAS in complex with GDP

Wild-type KRAS(1-166)-GMPPNP complex at 1.35 Ångstroms

Full-length Wild-type KRAS-GDP complex at 1.6 Ångstroms

Switch-I
Switch-II
P-loop
Extended switch-I conformation in KRAS

• Validate presence of extended switch-I conformation in solution by NMR.
    
    *Que Van at FNLCR*
  – High-pressure NMR studies in collaboration with Dr. Kalbitzer, University of Regensburg, Germany.

• Virtual compound screening to target the groove present at the base of switch-I region
  – in collaboration with Dr. Brian Shoichet’s group at UCSF.
 KRAS Effector Signaling: An extensive and complex network

Direct KRAS effectors

MAPK
  - RAF
  - MEK
  - ERK

PI3K
  - PI3K
  - AKT
  - ROCK

RHO
  - p190
  - RHO

RAC
  - TIAM1
  - RAC

RAL
  - RaIGEF
  - RaA
  - RaB
  - PLCε

PLCε

Indirect KRAS effectors

Survival
- Metabolism
- Autophagy
- Epigenetics
- Stress

⇒ 40 KRAS Effector Nodes

GEFs

GAPs
Complete NODE knockdown: compensatory activation by redundant isoforms masks the importance of many nodes

→ 40 KRAS Effector Nodes = 84 genes

Christof Fellmann, Scott Lowe, Chih-Shia Lee, Ji Luo
Effector Dependency Profile

<table>
<thead>
<tr>
<th>Effect</th>
<th>Cell viability</th>
<th>Proliferation</th>
<th>ROS levels</th>
<th>Apoptosis</th>
<th>Cell size</th>
</tr>
</thead>
</table>

**K-Ras Effector NODE:** 1 2 3 4 5 6 7 8 9 10 . . . . . . . . . . . . . . . . . . . 35 36 37 38 39 40

Cellular response

0% 0% Node knockdown 100%
Global assessment of KRAS-effector dependency

Tissue Origin
- lung
- pancreas
- large intestine
- colon

Sensitive
Resistant

p-value = 4.842e-06

* AUC computed by Ming Yi

Arnaud Amzallag
stine
Lung_NSCLC
Pancreas
Engineering baculovirus for improved production of processed KRAS

- recombineering used to insert FNTA/FNTB genes into the baculovirus genome
- eliminated issues with coinfection of multiple viruses
- maltose-binding protein (MBP) fusion for greater yield and solubility
- *Trichoplusia ni* (Hi5) insect cells for increased yield
Processed KRAS4b characterization

- Extensive protein characterization
  - Purified to homogeneity; yield >7mg/L
  - Intact mass
  - Predominantly monomeric
  - Secondary structure equivalent to non-processed KRAS4b
  - Lower thermal stability

Intact mass analysis

Analytical ultracentrifugation

Secondary structure by CD

Bill Gillette, Zhaojing Meng, Shelley Perkins, Peter Frank, Pat Alexander, Rodolfo Ghirlando
KRAS4b-FME binds to CRAF-RBD on Nanodiscs

Matt Holderfield, Maria Abreu Blanco
RAS Localization Assay Overview

60 Wells

~125 Cells/location

Hit identification

8 Locations/well

Segmentation and Analysis

GFP-KRAS4b^{G12V}

Nucleus  GFP  Membrane

Alla Brafman
NCI Developmental Therapeutics Program screening set

Primary assay: GFP-KRAS$^{G12V}$

~800 small molecules with biological activity

Reconfirmed hits
HaloTag-KRAS4b can be imaged in live cells. HaloTag-KRAS4b rescues RASless MEF proliferation.

Untreated MEFs
G1 arrest (day 19*)
+4-OHT
+HaloTagKRAS

Cell permeant, super bright, fluorescent Halo ligand from Janelia Farms

TIRF Image: membrane
Transmitted light image

Scale bar 20 µm

HRAS<sup>−/−</sup> NRAS<sup>−/−</sup> KRAS<sub>lox/lox</sub> MEFs

Drosten M, Dhawahir A, Sum EY, Urosevic J, Lechuga CG, Esteban LM, Castellano E, Guerra C, Santos E, Barbacid M.
EMBO J. 2010
Characterization of RAS molecules in live cell membranes

Jump squared displacement analysis

HaloTag-KRAS\textsuperscript{WT} driven-MEFs

Three components

<table>
<thead>
<tr>
<th>Model</th>
<th>Diffusion (um\textsuperscript{2}/s)</th>
<th>Fraction Mean (SDev)</th>
<th>Const. Rad. R\textsubscript{c} (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 → Normal</td>
<td>0.73</td>
<td>0.505 (0.0193)</td>
<td>-</td>
</tr>
<tr>
<td>2 → Constrained</td>
<td>0.1805</td>
<td>0.233 (0.021)</td>
<td>44.2</td>
</tr>
<tr>
<td>3 → Constrained</td>
<td>0.0178</td>
<td>0.2624 (0.026)</td>
<td>1.2</td>
</tr>
</tbody>
</table>

De Chen and Prabhakar Gudla
Single molecule tracking analysis suggests three RAS states in live cell membranes.

Information extracted from individual trajectories

Hypothesis: states represent different complexes in membrane.

RASless-MEFs, HaloTag-wtKRAS4b [JF646]=50pM, Serum Starved, 37°C, 22,325 trajectories and average trajectory length 12 frames.

De Chen and Prabhakar Gudla
### Collaboration with the RAS Community

**RAS events**
- Synthetic Lethality Workshop, January 6-7 2014
- RAS Pathways Workshop, June 11, 2014
- Cell Surfaces Workshop, July 23, 2014
- AACR Annual Meeting, April 21, 2015
- RAS Structures Workshop, July 21-22, 2015
- RAS Immunotherapy Workshop, November 3, 2015
- RAS Symposium, December 15-16, 2015

**Seminars at FNLCR**
- Channing Der, UNC
- Ken Westover, UTSW
- Carla Mattos, Northeastern
- Mark Philips, NYU
- Vadim Gaponenko, U-Chicago
- Josh Salafsky, Biodesy, Inc.
- Calvin Kuo, Stanford
- Kris Wood, Duke
- Mariano Barbadic, CNIO, Madrid
- Cyril Benes, Mass General
- Carolyn Buser, GlaxoSmithKline
- Jay Groves, UC-Berkeley
- Stephen Sligar, UI-Champaign Urbana
- Raffit Hassan, NCI
- Renata Grifantini, Extemautics Spa, Siena
- Renata Pasqualini, U-New Mexico
- Andrew Bradbury, Los Alamos
- Kent Rossman, UNC
- Shiva Malek, Genentech

**Outside collaborators**
- Steve Almo, Einstein
- Jim Wells, USCF
- Channing Der, UNC
- Ken Westover, UTSW
- Carla Mattos, Northeastern
- Steve Sligar, UI-Ill
- Jay Groves, Berkeley
- Hirsch Nanda, Susan Kreuger, NIST
- John Markley, NMRFAM, UW-Madison
- Paul Cohen, DARPA
- Kris Wood, Duke
- David Weber, U-Maryland
- Tina Yuan, Broad
- Cameron Pitt, UCSF
- Krishna Kota, USAMRIID
- Sotirios Koutsopoulos, MIT
- Fred Wittinghofer, Dortmund University
- Lynn McGregor, UCSF (PanCan postdoc)
- John Hunter, UTSW (PanCan postdoc)
- Saori Sato, Daiichi-Sankyo
- Walter Englaro, Sanofi-Aventis
- Kirk Staschke, Lilly
- Gad Getz, Mass Gen /Broad
- Matt Meyerson, Dana Farber

**Immunovo-MRM of RAS pathway**
- Amanda Paulovich, Fred Hutch
- Steve Carr, Broad Institute
- John Koomen, Moffit Cancer Center
- Andreas Gosberg, Lilly
Collaboration with the RAS Community

**RAS Reference Reagents**
- Chris Kemp, Fred Hutch
- Eric Chang, Baylor
- Silvia Thöne, Munich
- Peter Jackson, Stanford University
- Tyler Jacks, MIT
- Calvin Kuo, Stanford
- Bill Hahn, Broad / Dana Farber
- Karla Satchell, Northwestern
- Julian Downward, Cancer Research UK
- Daniel Abankwas, University of Turku
- Said Sebti, Moffitt Cancer Center
- Ian Prior, Liverpool
- Muller Fabbri, Children’s Hospital LA
- Faraz Bishehsari, Rush
- Amy Lee, USC
- Yosef Yarden, Weizmann
- Richard Klemke, UCSD
- Saidul Chowdhury, U-Texas Arlington
- Christian Gocke, JHMI
- Tobias Baumgart, U-Penn
- Emil Lou, U-Minnesota
- Ron Bose, Wash U
- Neil Kelleher, Northwestern
- Sourav Bandyopadhyay, UCSF
- Robert Chapkin, Texas A&M

**NIH collaborators**
- Ji Luo, NCI
- Anton Simeonov, NCATS
- Debbie Morrison, NCI
- Rajat Varma, NIAID
- Udo Rudloff, NCI
- Sriram Subramaniam, NCI