RAS oncogene
Research goals

Our research goals

• Understand mechanism of KRAS-driven oncogenesis
• Identify better therapeutic strategies for KRAS mutant tumors

Clinical Challenge
• ~ 200,000 new patients / year
• No effective targeted therapies
Outline

**Lecture Outline**

1. Discovery of the Ras oncogene
2. Ras signaling & oncogenesis
3. Targeting Ras and its signaling network
4. Synthetic lethal partners of the KRAS oncogene
5. Identify optimal target combinations for KRAS mutant cancer cells
6. Concluding thoughts
RAS oncogene

Discovery of the Ras Oncogene in Murine Tumor Viruses

<table>
<thead>
<tr>
<th>Year</th>
<th>Reference</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1964</td>
<td>Harvey (Nature, 1964)</td>
<td>An Unidentified virus which causes the rapid production of tumors in mice</td>
</tr>
<tr>
<td>1967</td>
<td>Kirsten &amp; Mayer (J. NCI, 1967)</td>
<td>Morphologic response to a murine erythroblastosis virus</td>
</tr>
</tbody>
</table>
# Molecular Characterization of the Viral Ras Protein

<table>
<thead>
<tr>
<th>Year</th>
<th>Reference</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>1979</td>
<td>Shih ... Scolnick (Virology, 1979)</td>
<td>Identification of a sarcoma virus-coded phosphoprotein in nonproducer cells transformed by Kirsten or Harvey murine sarcoma virus.</td>
</tr>
<tr>
<td></td>
<td>Scolnick... Shih (PNAS, 1979)</td>
<td>Guanine nucleotide-binding activity as an assay for src protein of rat-derived murine sarcoma viruses.</td>
</tr>
</tbody>
</table>
Mammalian counterpart

Viral Ras Gene Has Mammalian Counterpart

<table>
<thead>
<tr>
<th>Year</th>
<th>Reference</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Santos ... Barbacid (Nature, 1982)</td>
<td>T24 human bladder carcinoma oncogene is an activated form of the normal human homologue of BALB- and Harvey-MSV transforming genes.</td>
</tr>
<tr>
<td></td>
<td>Parada ... Weinberg (Nature, 1982)</td>
<td>Human EJ bladder carcinoma oncogene is homologous of Harvey sarcoma virus ras gene.</td>
</tr>
<tr>
<td></td>
<td>Der ... Cooper (PNAS, 1982)</td>
<td>Transforming genes of human bladder and lung carcinoma cell lines are homologous to the ras genes of Harvey and Kirsten sarcoma viruses.</td>
</tr>
</tbody>
</table>
### Human Ras Oncogene Has a Point Mutation

<table>
<thead>
<tr>
<th>Year</th>
<th>Reference</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1982</td>
<td>Tabin ... Weinberg (Nature, 1982)</td>
<td>Mechanism of activation of a human oncogene</td>
</tr>
<tr>
<td>1982</td>
<td>Reddy ... Barbacid (Nature, 1982)</td>
<td>A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene</td>
</tr>
<tr>
<td>1982</td>
<td>Toporowsky ... Wigler (Nature, 1982)</td>
<td>Activation of the T24 bladder carcinoma transforming gene is linked to a single amino acid change.</td>
</tr>
<tr>
<td>1982</td>
<td>Copon ... Goeddel (Nature, 1982)</td>
<td>Activation of Ki-ras2 gene in human colon and lung carcinomas by two different point mutations</td>
</tr>
</tbody>
</table>

**Ras gene in human cancer cells**
Transforming oncogene

Human Ras Oncogene Encodes a Transforming Oncoprotein

1984

Stacey ... Kung (Nature, 1984) Transformation of NIH 3T3 cells by microinjection of Ha-ras p21 protein

1985

Feramisco ... Sweet (Cell, 1984) Microinjection of the oncogene form of the human H-ras (T-24) protein results in rapid proliferation of quiescent cells

Mulcahy ... Stacey (Nature, 1985) Requirement for ras proto-oncogene function during serum-stimulated growth of NIH 3T3 cells

Ras

Neoplastic transformation
Biochemical properties

### Biochemical Properties of the Ras Protein

<table>
<thead>
<tr>
<th>Year</th>
<th>Authors</th>
<th>Publication</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1984</td>
<td>Sweet et al.</td>
<td><em>Nature</em>, 1984</td>
<td>The product of ras is a GTPase and the T24 oncogenic mutant is deficient in this activity.</td>
</tr>
</tbody>
</table>

*GTPase*: 
- **Ras GTPase**: Molecular switch 
- **GAP**
Ras family
Ras GDP-GTP Cycle
Ras membrane localization
Ras signaling network
Ras mutations

Incidence of Ras mutations in human cancer

<table>
<thead>
<tr>
<th>Cancer</th>
<th>% KRAS</th>
<th>% NRAS</th>
<th>% HRAS</th>
<th>% all Ras</th>
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</thead>
<tbody>
<tr>
<td>Pancreatic ductal adenocarcinoma</td>
<td>93.7</td>
<td>0.0</td>
<td>0.0</td>
<td>93.7</td>
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<tr>
<td>Colorectal adenocarcinoma</td>
<td>44.7</td>
<td>1.5</td>
<td>0.0</td>
<td>46.2</td>
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<tr>
<td>Multiple myeloma</td>
<td>21.8</td>
<td>19.6</td>
<td>0.0</td>
<td>41.4</td>
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<tr>
<td>Lung adenocarcinoma</td>
<td>30.9</td>
<td>0.3</td>
<td>0.0</td>
<td>31.2</td>
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<tr>
<td>Bladder cancer</td>
<td>0.0</td>
<td>2.0</td>
<td>0.0</td>
<td>2.0</td>
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<tr>
<td>Urothelial carcinoma</td>
<td>21.4</td>
<td>0.8</td>
<td>0.0</td>
<td>22.2</td>
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<tr>
<td>Uterine cervical carcinoma</td>
<td>12.5</td>
<td>0.8</td>
<td>0.0</td>
<td>13.3</td>
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<tr>
<td>Thyroid carcinoma</td>
<td>4.0</td>
<td>0.8</td>
<td>0.0</td>
<td>4.8</td>
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<tr>
<td>Acute myeloid leukemia</td>
<td>5.1</td>
<td>0.7</td>
<td>0.0</td>
<td>5.8</td>
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<tr>
<td>Melanocytic skin carcinoma</td>
<td>5.1</td>
<td>0.4</td>
<td>0.0</td>
<td>5.5</td>
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<tr>
<td>Carcinoma of head and neck</td>
<td>11.4</td>
<td>0.9</td>
<td>0.0</td>
<td>13.3</td>
</tr>
<tr>
<td>Conventional melanoma</td>
<td>5.7</td>
<td>0.0</td>
<td>0.0</td>
<td>5.7</td>
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<tr>
<td>Basal cell carcinoma</td>
<td>0.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.5</td>
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<tr>
<td>Diffuse large cell lymphoma</td>
<td>0.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.5</td>
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<tr>
<td>Oropharyngeal carcinoma</td>
<td>1.8</td>
<td>0.0</td>
<td>0.0</td>
<td>1.8</td>
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<tr>
<td>Chronic myelogenous leukemia</td>
<td>1.9</td>
<td>0.0</td>
<td>0.0</td>
<td>1.9</td>
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<tr>
<td>Urothelial cell carcinoma</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
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<tr>
<td>Small cell lung carcinoma</td>
<td>1.4</td>
<td>0.0</td>
<td>0.0</td>
<td>1.4</td>
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<tr>
<td>Renal papillary cell carcinoma</td>
<td>1.3</td>
<td>0.0</td>
<td>0.0</td>
<td>1.3</td>
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<tr>
<td>Adrenal gland carcinoma</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>Choroid plexus carcinoma</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>Hepatocellular carcinoma</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>Breast invasive carcinoma</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Colorectal adenocarcinoma</td>
<td>1.7</td>
<td>0.0</td>
<td>0.0</td>
<td>1.7</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td>0.6</td>
<td>0.0</td>
<td>0.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Adrenocortical carcinoma</td>
<td>1.1</td>
<td>0.0</td>
<td>0.0</td>
<td>1.1</td>
</tr>
</tbody>
</table>

(From NCI Ras Initiative)
(Cos et al, Nature Review Drug Discovery 2014)
Mutation spectrum

Spectrum of Ras Mutations in Human Cancer

Frequency of aa mutation across Ras paralogs

(Cox et al., Nature Review Drug Discovery 2014)
GTP hydrolysis
**Oncogenic driver**

### KRAS is a Major Oncogenic Driver in Adenocarcinomas

<table>
<thead>
<tr>
<th></th>
<th>Early Neoplasia</th>
<th>Adenoma</th>
<th>Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lung</strong></td>
<td>KRAS</td>
<td>KRAS TP53</td>
<td>KRAS TP53 LKB1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>...</td>
</tr>
<tr>
<td><strong>Pancreas</strong></td>
<td>KRAS</td>
<td>KRAS CDKN2A</td>
<td>KRAS CDKN2A TP53</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>...</td>
</tr>
<tr>
<td><strong>Colon</strong></td>
<td>APC</td>
<td>APC KRAS</td>
<td>APC KRAS TP53</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>...</td>
</tr>
</tbody>
</table>
Neoplastic transformation
Ras signaling
Ras membrane association

- Alternative membrane localization pathway
- Lack of KRAS selectivity – pathway shared by other small GTPases
Ras inhibition

Direct Inhibition of Ras Oncoprotein Function

- GTP binding affinity very high
- KRAS-G12C covalent inhibitors
  - ARS-853

KRAS-GDP ↔ KRAS-GTP

Large, flat P-P interaction surface
Direct inhibition
Kinase inhibitors
MAPK pathway

MAPK Pathway is Essential for Ras-Driven Cell Proliferation

- Ras WT cells
  - Proliferation: ✔️ ✔️ ✔️

- Ras-less cells
  - Proliferation: ✗

- Ras-less cells
  - RAF* / MEK* / ERK*
    - Proliferation: ✔️

- Ras-less cells
  - PI3K*
    - Proliferation: ✗

- Ras-less cells
  - Ral*
    - Proliferation: ✗

- Ras-less cells
  - RAF* + PI3K* + RalGD5*
    - Proliferation: ✔️ ✔️ ✔️

(Drosten et al., EMBO J., 2010)
MAPK pathway

RAF Inhibitors Activates MAPK Pathway in Ras-Mutant Cells
Feedback activation
MEKi combinations

Current MEKi Combinations Are Ineffective in KRAS Mutant Cancer

SELECT-1 Trial: advanced NSCLC Tumors with KRAS mutation

Combination of targeted therapy
- MEKi + PI3KI
- MEKi + EGFRi
- MEKi + mTOR
- MEKi + CDK4/6

Toxicity?
Therapeutic window

Therapeutic Window is Key to Effective Combination Therapy in Cancer

Good combination therapies

- Confer genotype-specific synergy
- Widens the therapeutic window
- Delays on-set of drug resistance
## Synthetic lethality

**Synthetic Lethality Reflects Genetic Buffering and Pathway Redundancy**

<table>
<thead>
<tr>
<th>Gene A</th>
<th>Gene B</th>
<th>Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>WT</td>
<td>✔️</td>
</tr>
<tr>
<td>WT</td>
<td>Loss</td>
<td>✔️</td>
</tr>
<tr>
<td>Mutant</td>
<td>WT</td>
<td>✔️</td>
</tr>
<tr>
<td>Mutant</td>
<td>Loss</td>
<td>✗</td>
</tr>
</tbody>
</table>
Oncogene addiction

KRAS Mutant Cells Exhibit Oncogene & Non-oncogene Addiction

Oncogenic Stress

Stress Response Pathways

Non-oncogene addiction

Luo et al., Cell 2009
Synthetic lethal interactions

Synthetic Lethal Interactions in KRAS Mutant Cells

- Tissue- and genetic context-driven synthetic lethal interactions
- Cooperate with KRAS oncogenic signaling pathways

(Yu & Luo, The Enzymes 2014)
Dissecting the contribution

**Dissecting The Contribution of Oncogene and Non-oncogene Addiction in KRAS Mutant Cancer**

- **What are the critical onco-effectors for mutant KRAS?**
  - Distinguishing oncogenic and physiological Ras signaling
  - Critical for mutant KRAS signaling, dispensable in normal cells

- **How is KRAS addiction communicated through its effector network?**
  - Partitioning of KRAS dependency among pathways
  - Interaction and cooperation among pathways

- **What are the critical stress-response pathways in KRAS mutant cells?**
  - Activated by oncogenic stress, dispensable in normal cells

- **What are the rational target combinations downstream of mutant KRAS?**
  - Genotype selectivity
  - Orthogonal mechanisms of action
siRNA platform

A Combinatorial siRNA Platform to Co-targeting Multiple Genes And Evaluate Target Combinations

(Yuan et al. Cancer Discovery 2014)
KRAS addiction
Ras effector and stress response

Mechanism of KRAS Addiction Through Ras Effector and Stress Response Pathways

Positive Ctrl
KRAS

Gene nodes
RAF
etc.

Node pairs
RAF + RAC
etc.

Gene combos
BRAF
CRAF
RAC1
etc.

10 KRAS mut CRC & PDAC cell lines
3 KRAS WT CRC & PDAC cell lines
4 Untransformed cell lines

(Lee et al. PNAS 2019)
Single node dependency

Single Node Dependency Profile in KRAS Mutant Cells

Differential Dependency Score (%)

\[ DDS = \frac{V_{\text{WT}} - V_{\text{mut}}}{V_{\text{WT}}} \]

Pearson correlation

\[ R = \text{corr}(\text{siKRAS}_{\text{mut}}, \text{siComb}_{\text{mut}}) \]

Average DD5 (%) (Lee et al. PNAS 2019)
Private node dependency

Private Node Dependency Reveals Heterogeneity in Pathway Utilization for Supporting KRAS Addiction

(From et al. PNAS 2019)
Paired node dependency
Paired node pair dependencies

Public Node Pair Dependencies in KRAS Mutant Cell Lines

(See et al. PNAS 2019)
Private node pair dependency

Private Node Pair Dependency Is Dictated by Private Single Node Dependency

(Lee et al. PNAS 2019)
Gene paralog combinations

Deconvolution of Gene Paralog Combinations

Differential Dependency Score (%)

\[ DDS = \frac{V_{\text{mut}} - V_{\text{WT}}}{V_{\text{WT}}} \]

Differential Dependency Score Normal Cells (%)

\[ DDSn = \frac{V_{\text{mut}}^n - V_{\text{WT}}^n}{V_{\text{WT}}^n} \]

Pearson correlation

\[ R = \text{corr}(\text{sIRAS}_{\text{mut}}, \text{sComb}_{\text{mut}}) \]

(Lee et al. PNAS 2019)
Deconvolution

Deconvolution of Gene Paralog Combinations

(Lee et al. PNAS 2019)
Knockdown

RAC1 and ATG7 Knockdown Sensitizes KRAS Mutant Cells Towards MAPK Pathway Inhibitors

RAF inhibitor RAF709

MEK inhibitor Trametinib

(Lee et al. PNAS 2019)
Cell cycle arrest

BRAF, CRAF and ATG7 Co-depletion Enhances Cell Cycle arrest and Cell death in KRAS Mutant Cells

(Lee et al. PNAS 2019)
Autophagy pathways
RAF kinase and autophagy
Co-targeting the MAPK and Autophagy Pathway In KRAS Mutant Pancreatic Cancer Cells

*Kinsey ... McMahon (Nature Medicine 2019)*
Protective autophagy elicited by RAF→MEK→ERK inhibition suggests a treatment strategy for RAS-driven cancers

*Bryant ... Der, (Nature Medicine 2019)*
Combination of ERK and autophagy inhibition as a treatment approach for pancreatic cancer

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**Mia-PaCa2 xenograft tumor**

![Graph showing tumor volume changes over treatment days for Mia-PaCa2 xenograft tumor.](image)

**KRAS mutant pancreatic cancer patient**

![Graph showing clinical trial outcomes for KRAS mutant pancreatic cancer patient.](image)

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**Phase I Trial: Trametinib and Hydroxychloroquine in Treating Patients With Pancreatic Cancer**
MAPK and autophagy

Co-targeting the MAPK and Autophagy Pathway In KRAS Mutant Pancreatic Cancer Cells

Kinsey ... McMahon (Nature Medicine 2019)
Protective autophagy elicited by RAF→MEK→ERK inhibition suggests a treatment strategy for RAS-driven cancers

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Mia-PaCa2 xenograft tumor

KRAS mutant pancreatic cancer patient

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