RAS oncogene

The Ras Oncogene

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NCI

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NIH NATIONAL CANCER INSTITUTE
Center for Cancer Research
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><em>Harvey (Nature, 1964)</em></td>
<td>An Unidentified virus which causes the rapid production of tumors in mice</td>
</tr>
<tr>
<td>1967</td>
<td><em>Kirsten &amp; Mayer (J. NCI, 1967)</em></td>
<td>Morphologic response to a murine erythroblastosis virus</td>
</tr>
</tbody>
</table>

![Diagram showing virus infection leading to tumor formation in a mouse]
Molecular characterization

Molecular Characterization of the Viral Ras Protein

<table>
<thead>
<tr>
<th>Year</th>
<th>Reference</th>
<th>Description</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>1980</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>
<tr>
<td>1980</td>
<td>Willingham ... Scolnick (Cell, 1980)</td>
<td>Localization of the src gene product of the Harvey strain of MSV to plasma membrane of transformed cells by electron microscopic immunocytochemistry</td>
</tr>
</tbody>
</table>
Mammalian counterpart

Viral Ras Gene Has Mammalian Counterpart

<table>
<thead>
<tr>
<th>Year</th>
<th>Reference</th>
<th>Description</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>
**Point mutations**

### Human Ras Oncogene Has a Point Mutation

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

![Ras gene in human cancer cells](image-url)
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 of the Ras Protein

<table>
<thead>
<tr>
<th>Year</th>
<th>Reference</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>1984</td>
<td>Sweet ... Lowy (Nature, 1984)</td>
<td>The product of ras is a GTPase and the T24 oncogenic mutant is deficient in this activity</td>
</tr>
<tr>
<td>1985</td>
<td>Gibbs ... Scoinick (PNAS, 1985)</td>
<td>Intrinsic GTPase activity distinguishes normal and oncogenic ras p21 molecules</td>
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<tr>
<td>1985</td>
<td>Hanne ... Kung (PNAS, 1985)</td>
<td>Ha-ras proteins exhibit GTPase activity: point mutations that activate Ha-ras gene products result in decreased GTPase activity</td>
</tr>
<tr>
<td>1987</td>
<td>Trahey ... McCormick (Science, 1987)</td>
<td>A cytoplasmic protein stimulates normal N-ras p21 GTPase, but does not affect oncogenic mutants</td>
</tr>
</tbody>
</table>

![Diagram of Ras GTPase and Molecular Switch](image.png)
Ras family

The Ras Family of Small GTPases

(Corr et al. Nature Reviews Drug Discovery 2014)
Ras GDP-GTP Cycle
Ras membrane localization
Ras signaling network
Incidence of Ras mutations in human cancer

Ras mutations occur in 10% of 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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<tbody>
<tr>
<td>Pancreatic ductal adenocarcinoma</td>
<td>91.7</td>
<td>3.6</td>
<td>4.7</td>
<td>91.7</td>
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<tr>
<td>Colorectal adenocarcinoma</td>
<td>44.7</td>
<td>7.7</td>
<td>6.4</td>
<td>59.8</td>
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<tr>
<td>Multiple myeloma</td>
<td>33.8</td>
<td>18.3</td>
<td>18.4</td>
<td>60.5</td>
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<tr>
<td>Lung adenocarcinoma</td>
<td>39.9</td>
<td>9.9</td>
<td>3.9</td>
<td>53.7</td>
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<tr>
<td>Bladder transitional cell carcinoma</td>
<td>8.8</td>
<td>5.0</td>
<td>0.5</td>
<td>14.3</td>
</tr>
<tr>
<td>Uterine corpus endometrial sarcoma</td>
<td>21.5</td>
<td>3.9</td>
<td>0.4</td>
<td>25.8</td>
</tr>
<tr>
<td>Uterine sarcoma</td>
<td>12.2</td>
<td>2.0</td>
<td>0.5</td>
<td>14.7</td>
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<tr>
<td>Thyroid carcinoma</td>
<td>1.0</td>
<td>0.5</td>
<td>0.3</td>
<td>1.5</td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
<td>5.1</td>
<td>0.7</td>
<td>0.2</td>
<td>6.0</td>
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<tr>
<td>Medullary thyroid carcinoma</td>
<td>3.1</td>
<td>0.4</td>
<td>0.0</td>
<td>3.5</td>
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<tr>
<td>Cervical adenocarcinoma</td>
<td>11.4</td>
<td>2.5</td>
<td>0.3</td>
<td>14.2</td>
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<tr>
<td>Colorectal adenocarcinoma</td>
<td>8.2</td>
<td>1.7</td>
<td>0.1</td>
<td>10.0</td>
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<td>Small intestine adenocarcinoma</td>
<td>4.5</td>
<td>0.3</td>
<td>0.2</td>
<td>5.0</td>
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<td>Diffuse large B-cell lymphoma</td>
<td>3.2</td>
<td>0.6</td>
<td>0.1</td>
<td>4.0</td>
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<tr>
<td>Esophageal adenocarcinoma</td>
<td>5.8</td>
<td>0.3</td>
<td>0.1</td>
<td>6.2</td>
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<tr>
<td>Chronic lymphocytic leukemia</td>
<td>1.0</td>
<td>2.5</td>
<td>0.0</td>
<td>3.5</td>
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<tr>
<td>Lymphoproliferative disease</td>
<td>2.2</td>
<td>0.5</td>
<td>0.0</td>
<td>2.7</td>
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<tr>
<td>Small cell lung carcinoma</td>
<td>1.4</td>
<td>2.5</td>
<td>0.0</td>
<td>2.2</td>
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<tr>
<td>Renal papillary carcinoma</td>
<td>1.0</td>
<td>0.2</td>
<td>0.0</td>
<td>1.2</td>
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<tr>
<td>Adrenal cortical carcinoma</td>
<td>0.6</td>
<td>0.2</td>
<td>0.0</td>
<td>0.8</td>
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<tr>
<td>Chromophobe renal cell carcinoma</td>
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<td>0.4</td>
<td>0.1</td>
<td>0.5</td>
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<td>Hepatocellular carcinoma</td>
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<td>0.1</td>
<td>0.0</td>
<td>1.2</td>
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<tr>
<td>Breast invasive carcinoma</td>
<td>0.7</td>
<td>0.4</td>
<td>0.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Colorectal adenocarcinoma</td>
<td>1.1</td>
<td>0.4</td>
<td>0.1</td>
<td>1.6</td>
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<tr>
<td>Adrenocortical carcinoma</td>
<td>1.1</td>
<td>0.4</td>
<td>0.1</td>
<td>1.6</td>
</tr>
</tbody>
</table>

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

Spectrum of Ras Mutations in Human Cancer

(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>Lung</td>
<td>KRAS</td>
<td>KRAS TP53</td>
<td>KRAS TP53</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LKB1 ...</td>
</tr>
<tr>
<td>Pancreas</td>
<td>KRAS</td>
<td>KRAS CDKN2A</td>
<td>KRAS CDKN2A</td>
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<td></td>
<td></td>
<td></td>
<td>TP53 ...</td>
</tr>
<tr>
<td>Colon</td>
<td>APC</td>
<td>APC KRAS</td>
<td>APC KRAS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TP53 ...</td>
</tr>
</tbody>
</table>
Neoplastic transformation

Oncogenic Ras Signaling Leads to Neoplastic Transformation

Ras

MAPK PI3K Rac Rho Ral PLC

Proliferation, survival, metabolism, motility, gene expression

Apoptosis Neoplasia Senescence Apoptosis

Epithelial monolayer Epithelial monolayer Epithelial monolayer

+ KRAS* − KRAS*

Oncogene addiction
Ras signaling
Ras membrane association

Inhibition of Ras Membrane Association

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

MAPK Pathway is Essential for Ras-Driven Cell Proliferation

- Ras WT cells
- Ras-less cells
- Ras-less cells with RAF*/MEK*/ERK*
- Ras-less cells with PI3K*
- Ras-less cells with RaI*
- Ras-less cells with RAF*/PI3K*/RaI/GDS*

Proliferation
- ✔️ ✔️ ✔️
- ❌
- ✔️
- ❌
- ❌
- ✔️ ✔️ ✔️

(Drosten et al., EMBO J., 2010)
RAF Inhibitors Activates MAPK Pathway in Ras-Mutant Cells

(Holderfield & Stuart, BJC, 2014)
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

<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
Synthetic lethal interactions

**Synthetic Lethal Interactions in KRAS Mutant Cells**

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

(Yo & 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
Single node dependency

**Single Node Dependency Profile in KRAS Mutant Cells**

Differential Dependency Score (DLS): $\text{DDS} = \bar{V}_{\text{mut}} - \bar{V}_{\text{wt}}$

Pearson correlation:

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

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

Public Node Pair Dependencies in KRAS Mutant Cell Lines

(Le 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 (%)

\[ DDS = \bar{V}_{\text{WT}} - \bar{V}_{\text{WT}} \]

Differential Dependency Score Normal Cells (%)

\[ DDS_n = \bar{V}_{\text{WT}} - \bar{V}_{\text{WT}} \]

Pearson correlation

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

(Lee et al. PNAS 2019)
Deconvolution
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

---

**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

Bryant ... Der, (Nature Medicine 2019)
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Phase I Trial: Trametinib and Hydroxychloroquine in Treating Patients With Pancreatic Cancer
Acknowledgment