Generating Conformation- and Mutation-Specific Antibodies to Disrupt Oncogenic RAS Signaling

Paul Marinec, Ph.D.
Wells Lab/UCSF
wellslab.ucsf.edu
Why RAS = Darth Vader

More than 30% of all human cancers are driven by mutations of RAS genes.

*Not a single therapeutic in the clinic that directly targets RAS
• acts as a binary switch, cycling b/t active & inactive states
• key controller of normal cellular growth & proliferation
• tightly regulated by GEFs and GAPs

Karnoub & Weinberg, 2008
Tight Regulation (Normally)

Inactivation involves GAPs, whereas activation involves PI3K and GEFs.
Mutations: Block Binding & ↓ Hydrolysis

Inactive

Unregulated Downstream Signaling

PI3K
Raf
RalGDS
TIAM-1
RASSF
AF6

Active

RasGAP
Cancers Exhibit Isoform + Mutation Specificity

<table>
<thead>
<tr>
<th></th>
<th>KRAS 12</th>
<th>KRAS 13</th>
<th>KRAS 61</th>
<th>HRAS 12</th>
<th>HRAS 13</th>
<th>HRAS 61</th>
<th>NRAS 12</th>
<th>NRAS 13</th>
<th>NRAS 61</th>
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<td>0.01</td>
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<td>0.02</td>
<td>0.11</td>
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<td>ALL</td>
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- RAS mutations occur in >90% of pancreatic carcinomas....no other human tumor comes close in mutational frequency

Adapted from the COSMIC Database
*33% of all human cancers carry a RAS mutation

*99.2% of all RAS mutations occur at codons 12, 13, or 61

*Point mutations result in 6 possible AAs at each position

<table>
<thead>
<tr>
<th>Gly12</th>
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<tr>
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</tr>
<tr>
<td>C</td>
<td>C</td>
<td>H</td>
</tr>
<tr>
<td>D</td>
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<td>K</td>
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<tr>
<td>R</td>
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<td>L</td>
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<tr>
<td>S</td>
<td>S</td>
<td>P</td>
</tr>
<tr>
<td>V</td>
<td>V</td>
<td>R</td>
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The Conformational Dynamics of RAS

RAS Effector Regions
Switch I
Switch II

Can I engineer an antibody to discriminate between the active and inactive conformations, and/or specifically recognize mutant forms of the RAS oncoprotein?

Inactive GDP-Bound
Our Antibody Discovery Pipeline

- **Selection Design**
- **Phage Display**
- **Primary Validation**
  - **Competition ELISAs**
  - **Clone Sequencing**
  - **Specificity Testing**
- **Secondary Validation**
  - **Affinity Testing**
  - **Large Scale Purification**
  - **Epitope Mapping**
- **Antigen Preparation**
  - **Competitive**
  - **Subtractive**

Goal = 1 mutation- and/or conformation-specific Fab that I could use as a scaffold for engineering others.
**Power In Numbers: Our Phage Display Library**

*Diversity primarily confined to CDR-L3 & H3

*H1 & H2 comprised of reduced binary code

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### Library CDR-L3 CDR-H1 CDR-H2 CDR-H3 Total Diversity

<table>
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<tr>
<th></th>
<th>D</th>
<th>F</th>
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<tr>
<td><strong>Library</strong></td>
<td>896</td>
<td>2 x 10^7</td>
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<tr>
<td><strong>Theoretical Diversity</strong></td>
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<td>64</td>
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<tr>
<td><strong>Total</strong></td>
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<td>256</td>
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<tr>
<td><strong>Diversity</strong></td>
<td>5 x 10^22</td>
<td>2 x 10^17</td>
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Phage Panning on our ‘Fab-uccino Machine’

40 Antigens

Binding Selection

Fab Phage Pool

Phage

Fab

Target

Amplification

E. coli host

Non-binding Phage Washed Away

Round 1 = 100nM
Round 2 = 50nM
Round 3 = 25nM
Round 4 = 10nM
Round 5 (-) = 100nM
A Synopsis of >9 Months in 1 Slide

- **Antigens**: 40
  - Purified & Biotinylated
  - Phage Display Library “F”
  - \(3.0 \times 10^{10}\)
  - in vitro selections
- **Single Colony Isolation**: 4,224
- **“Specific” Binders**: 624
  - Competition ELISAs
  - Phage Amplification & Sequencing
- **Unique Clones**: 159
  - Specificity ELISAs
- **Non-specific Binders**: 129
  - Specificity ELISAs
- **Unique & Specific Binders**: 30
Determining Fab Affinities on the OctetRed384

- 6 Biosensors = Anti-Human Fab CH1 (FAB)
- $[\text{Ag}] = 250\text{nM} - 0.1\text{nM} + \text{Blank}$
- Association = 15min, Dissociation = 10-60min
- Regen: (10s in 10mM Glycine, 10s in buffer) 3X

### Mean $K_D$: 13.1nM, Range: 284pM - 28.9nM
**Classical Sandwich Binning: Pairwise**

1. Capture Target Fab
2. Load KRAS
3. Test other clones

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**Raw Data (Sensor Location)**

- Time (s): 0 to 16000
- Sensor Locations: A1, B1, C1, D1, E1, F1, G1, H1
Sandwich Binning: Pairwise Matrix

- ≥ 4 separate epitope bins present within this panel of Fabs
‘Functional’ Epitope Binning

- 7 Fabs blocked by Raf1-RBD
- 6 Fabs could bind elsewhere on RAS in the presence of the Raf1-RBD


Fabs blocked by Raf1 binding induced cell death after 24h in H1792 cells when transferred into a CP-scaffold
Summary

• RAS is an important oncology target

• BLI is a fantastic high-throughput platform for validating antibody discovery efforts

• ‘Functional Epitope Binning’ greatly accelerates the triage of lead candidates

• The Octet Red384 is AWESOME!
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Rashi Takkar

RECOMBINANT ANTIBODY NETWORK

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