Understanding Signaling Pathways by Modifying Sensitivity to PLX4720 in B-RAF$^{V600E}$ Melanoma

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What Is Melanoma?

- Leading cause of skin cancer death
- Originates in melanocytes
- Melanocytes produce skin/hair pigmentation

BRAF\textsuperscript{V600E} Mutation

- Mitogen-activated protein kinase (MAPK) is activated through phosphorylation of MEK $\rightarrow$ ERK $\rightarrow$ cell proliferation/growth
- B-RAF gene: proto-oncogene
- Gets mutated at codon 600
- 50-70% of metastatic melanomas harbor this mutation
- Mutant B-RAF constitutively activates the MAPK pathway
- Inhibiting B-RAF with PLX4720, the MAPK pathway is turned off and tumors regress
- Despite initially regressing, all tumors eventually developed drug resistance
A Screen for Kinases that Bypass B-RAF Inhibition

How Do Candidate Genes Induce Resistance?

Prioritization Screen (2 cell lines, 8-point GI₅₀)

<table>
<thead>
<tr>
<th>Rank</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>COT</td>
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<tr>
<td>2</td>
<td>C-RAF</td>
</tr>
<tr>
<td>3</td>
<td>CRKL</td>
</tr>
<tr>
<td>4</td>
<td>FGR</td>
</tr>
<tr>
<td>5</td>
<td>PRKCE</td>
</tr>
<tr>
<td>6</td>
<td>PRKCH</td>
</tr>
<tr>
<td>7</td>
<td>ERBB2</td>
</tr>
<tr>
<td>8</td>
<td>AXL</td>
</tr>
<tr>
<td>9</td>
<td>PAK3</td>
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</table>

Resistance via MAPK pathway activation

Cell Lines: SKMEL28 and A375 (B-RAF Mutant)
Hypothesis: Based on the results obtained from the screen, we hypothesized that PRKC ε, η, and θ reactivate the MAPK signaling pathway OR an alternative signaling pathway independent of the MAPK pathway.

PLX-4720
Understanding PKC Mediated Drug Resistance: Experimental Approach

Candidate Based Approach

Unbiased Approach
Experimental Approach

Candidate Based Approach

Western Blot

A375, malignant melanoma
BRAF^{V600E} mutation

http://coxsackie-virus.com/
Checking Alternative Signaling Pathways via Western Blotting

Major signaling pathways that are deregulated in melanoma

Checking Alternative Signaling Pathways via Western Blotting

Pathways/Genes:
- MAPK
- Cyclin D1
- SRC
- MARCKS
- STAT3 (Tyr705)
- ERBB2
- AKT
- m-TOR
- STAT3 (Ser727)

- Actin
- JNK
- NFκB
- RSK
- SMAD 2/3
- 70S6K
- CRAF
PRKC ε, η and θ Does Not Activate the MAPK pathway

<table>
<thead>
<tr>
<th></th>
<th>LacZ</th>
<th>PKCE</th>
<th>PKCH</th>
<th>PKCQ</th>
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<tbody>
<tr>
<td>+ PLX4720</td>
<td>-</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>- no PLX4720</td>
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<table>
<thead>
<tr>
<th>Protein</th>
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<tr>
<td>Total-ERK</td>
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<tr>
<td>Phospho-ERK</td>
<td>[Image]</td>
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<tr>
<td>Total-MEK</td>
<td>[Image]</td>
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<tr>
<td>Phospho-MEK</td>
<td>[Image]</td>
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</table>
### Checking Alternative Signaling Pathways via Western Blotting

**Pathways/Genes:**

- **MAPK**
  - Cyclin D1
  - SRC
  - MARCKS
  - STAT3 (Tyr705)
  - ERBB2
  - AKT
  - m-TOR
  - STAT3 (Ser727)

- **Actin**
- **JNK**
- **NFκB**
- **RSK**
- **SMAD 2/3**
- **70S6K**
Checking Alternative Signaling Pathways via Western Blotting

- Phospho-STAT3
- Total-STAT3
- Phospho-JNK

- PLX4720
- no PLX4720

- Phospho-AKT
- Total-AKT
- Phospho-S6
- Total-S6
Checking Alternative Signaling Pathways via Western Blotting

Pathways/Genes:
- Cyclin D1
- MAPK
- SRC
- MARCKS
- STAT3 (Tyr705)
- AKT
- m-TOR
- STAT3 (Ser727)
- Actin
- JNK
- NFκB
- RSK
- SMAD 2/3
- 70S6K
Experimental Approach

Unbiased Approach

Luminex Phosphotyrosine Kinase Profiling
### Luminex Phospho-Tyrosine Kinase Profiling

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<thead>
<tr>
<th>PLX:</th>
<th>LacZ</th>
<th>PKC-E</th>
<th>PKC-H</th>
<th>PKC-Q</th>
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#### Fold-Change (LacZ/DMSO):

- **ERBB2**
- **ERBB3**
- **ERBB4**
- **ERBB3**
- **MEK1/2**
- **RPS6KA1/3**
Luminex Phospho-Tyrosine Kinase Profiling...

Fold-Change (LacZ/DMSO): Low phosphorylation → High Phosphorylation

ERBB2 → ERBB3 → ERBB4
A Screen for Kinases that Bypass B-RAF Inhibition
Confirming Luminex Phospho-Tyrosine Kinase Profiling

- Sustained pERBB2 levels induced by PKC isoforms in the presence of PLX4720 may indicate that PKC mediates drug resistance through ERBB2. Further experiments will need to be done to validate this hypothesis.

- ERBB2 can also become activated through growth factors, further experiments need to be done to determine if external signaling is responsible for ERBB2 phosphorylation in PKCs.

- Future studies will investigate the source of resistance to PLX470 to promote long-term treatment strategies and accelerate the personalization of medicine for the treatment of melanoma.
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• Levi Garraway and Todd Golub

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• Broad Institute

• Cancer Program