Insights from Sequencing the Melanoma Exome

Michael Krauthammer, MD PhD, December 2 2015
2012 Exome Screens and Results

Exome Sequencing of 108 sun-exposed melanomas

--RAC1 Pathway: Discovery of $\text{RAC1}^{P29S}$, 3rd most frequent activating mutation in melanoma

-Aurora A Pathway: Recurrent inactivating mutations in $\text{PPP6C}$

-Inactivating NF1 mutation: 30% of BRAF/RAF WT patients
Exome Sequencing of **213 sun-exposed melanomas**, **2<sup>nd</sup> largest screen to date**

- 1) Comprehensive melanoma driver gene list

- 2) NF1 mutations co-occur with RASopathy mutations
Identifying Melanoma Driver Genes: How Many Exomes are Needed?

Lawrence et al., Nature, 2013
# Top Melanoma Drivers / Gene Mutation Burden

## 610 melanomas: Yale+TCGA+Broad

<table>
<thead>
<tr>
<th>Symbol</th>
<th><strong>Recurrent</strong></th>
<th>Incidence</th>
<th><strong>Inactivating</strong></th>
<th>Incidence</th>
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<tbody>
<tr>
<td></td>
<td><strong># samples</strong></td>
<td><strong>%</strong></td>
<td><strong># samples</strong></td>
<td><strong>%</strong></td>
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<tr>
<td><em>BRAF</em> 1</td>
<td>307</td>
<td>50.3</td>
<td><em>NF1</em> 1</td>
<td>63</td>
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<td>155</td>
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<td><em>ARID2</em></td>
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<td><em>CDKN2A</em></td>
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<tr>
<td>MAP2K1 1</td>
<td>18</td>
<td>3.0</td>
<td><em>TP53</em> 2 5</td>
<td>44</td>
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<td>IDH1 6</td>
<td>16</td>
<td>2.6</td>
<td><em>PTEN</em> 1</td>
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<tr>
<td>PPP6C 4</td>
<td>15</td>
<td>2.5</td>
<td><em>RB1</em> 1</td>
<td>19</td>
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<tr>
<td>TRRAP 4</td>
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<td>2.0</td>
<td><em>KMT2B</em></td>
<td>18</td>
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<tr>
<td>RQCD1 2</td>
<td>11</td>
<td>1.8</td>
<td><em>NOTCH2</em> 3</td>
<td>15</td>
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<tr>
<td>PCDHGA 13</td>
<td>11</td>
<td>1.8</td>
<td><em>ASPM</em> 4</td>
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<tr>
<td>BCL2L12 5</td>
<td>10</td>
<td>1.6</td>
<td><em>ARID1B</em> 2</td>
<td>13</td>
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<tr>
<td>DGKI 6</td>
<td>9</td>
<td>1.5</td>
<td><em>ARID1A</em> 2</td>
<td>13</td>
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</table>

1 MAPK/cell cycle progression  
2 Chromatin remodeling/DNA repair/transcription  
3 Intercellular signaling/cell-cell interaction  
4 Mitotic spindle  
5 Apoptosis  
6 Metabolism
BRAF/RAS/NF1 – Yale Cohort (n=213)

Krauthammer et al., 2015, Nature Genetics, July 2015
NF1 (Neurofibromin) - RAS GTPase-activating Protein

- 12% of Yale melanoma patients
- Mostly nonsense mutations, with loss of WT allele
- Often bi-allelic mutations

Red: nonsense; Brown: InDels; Gray: missense (damaging and tolerated)
NF1 Mutations Lead to Low NF1 Protein Expression, Variable NRAS Activation
NF1-Mutant Melanomas: MEK Inhibition

Diagram showing the pathway from NF1 to BRAF to MEK, with MEKi inhibition. The graph illustrates the effect of Selumetinib on NF1 mutants at different concentrations.
NF1 and Concurrent MAPK Pathway Mutations

Melanoma Group

<table>
<thead>
<tr>
<th></th>
<th>BRAF</th>
<th>RAS</th>
<th>NF1</th>
<th>WT</th>
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<tr>
<td>BRAF</td>
<td>91</td>
<td>1</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>NRAS</td>
<td>2</td>
<td>60</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NF1</td>
<td>9</td>
<td>3</td>
<td>33</td>
<td>0</td>
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<tr>
<td>TNC</td>
<td>10</td>
<td>5</td>
<td>28</td>
<td>5</td>
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<tr>
<td>RASA2</td>
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<td>0</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>TACC2</td>
<td>13</td>
<td>13</td>
<td>32</td>
<td>4</td>
</tr>
<tr>
<td>PPP6C</td>
<td>14</td>
<td>8</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PLEKHH2</td>
<td>3</td>
<td>2</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>PPP2R2B</td>
<td>1</td>
<td>0</td>
<td>8</td>
<td>0</td>
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</table>
RASA2 Mutations in NF1-mutant Melanomas

Yale Cohort

- 9 mutations in 7 NF1-mutant melanomas
  - 4 melanomas in LOH across the RASA2 locus
  - 2 melanomas are compound heterozygotes

- One recurrent mutations at position R511C in 3 NF1-mutant melanomas.

Compare to TCGA

R511C
RASA2 R511C Mutations in Noonan Syndrome (RASopathy)

- RASA2$^{R511C}$ has recently been found in a patient with Noonan syndrome, a RASopathy
- RASA2$^{R511C}$ increases p-ERK

Chen et al., PNAS 2014
Is there a link to germline RASopathy mutations?

RASopathies are caused by germline mutations in genes coding for transducers and modulator proteins participating in the RAS-MAP kinase (MAPK) signaling pathway

- Heart defects, spine problems, bleeding disorders etc.
- Incidence (Noonan syndrome): 1:1000 to 1:2500

- Noonan syndrome: RASA2, PTPN11, SOS1, RAF1
- Leopard syndrome: PTPN11
- Legius syndrome: SPRED1
Comparison of MAPK Pathway Gene Mutations in Melanoma and RASopathies

Melanoma
1989 NRAS/KRAS
2002 BRAF
2006 KIT
2009 MAP2 K1
2012 NF1/RAC 1

RASopathies
1990 NF1
2001 PTPN1
2006 KRAS/BRAF/MAP2
2007 SOS1/RAF1/SPRED
2009 SHOC2
2010 CBL/NRAS
2014 RASA2
Comparison of MAPK Pathway Gene Mutations in Melanoma and RASopathies

**Melanoma**
- NRAS/ KRAS 1989
- BRAF 2002
- 2006 KIT
- 2009 MAP2 K1

**RASopathies**
- NF1 1990
- PTPN1 1 2001
- 2006 KRAS/ BRAF/ MAP2 K
- SOS1/ RAF1/ SPRED 1 2007
- SHOC 2 2009
- 2010 CBL/ NRAS
- 2014 RASA2
- 2015 PTPN11 SOS1 RAF1 SPRED1 RASA2
NF1-mutant Melanomas and RASopathy gene mutations

- 60% of NF1-mutant melanomas have mutations in PTPN11, SOS1, RASA2, SPRED1 and RAF1
- The majority of mutations are documented disease causing
- Only 10% of the non-NF1 melanomas have mutations in these genes
Distribution of PTPN11 Mutations in RASopathies and Cancer

Noonan/Leopard Syndromes (germline):

TCGA (somatic):

Observed in NF1-mutant melanomas

Kontaridis et al., JBC, 2006
Clinical Evidence for Additive Effect of NF1 & PTPN11 Co-Mutations

- Clinical evidence for additive effects of NF1 and PTPN11 Mutations
  - Patients with germline NF1 and PTPN11 mutations have severe/lethal forms of Noonan S./Neurofibromatosis

Thiel et al., AJMG, 2009
Emerging Melanoma Mutational Landscape

**MAPK Pathway**
- RTK
- SOS1
- PTPN11
- SPRED1
- NF1
- RAS
- RASA2
- RAC1
- BRAF
- RAF1
- MEK/ERK
- P29S
- InDels, Stop, Splice
- Q61*, G12/13*
- V600*

**Cell Cycle Control**
- RB1
- CDKN2A

**AKT Pathway**
- PTEN

**Chromatin Interaction / DNA damage**
- PPP6C
- TERT
- TP53
- ATM

**Histone Methyltransferases**
- EZH2
- SETD2
- MLL1

**SWI/SNF**
- ARID2
- ARID1A
- ARID1B
From Whole Exome to Targeted Gene Sequencing

- Patient targeted sequencing to assist in melanoma
  - Diagnosis
  - Prognosis
  - Treatment prediction
- Goal: set of 100-200 melanoma driver genes (“what constitutes a melanoma?”)
- Include: Actionable mutations (NCI MATCH trial)

Table S5: OCP targeted alterations with approved and investigational targeted therapies

<table>
<thead>
<tr>
<th>Alteration</th>
<th>Indication</th>
<th>Example Treatment(s)</th>
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<tbody>
<tr>
<td>ALK fusion</td>
<td>Non-Small Cell Lung Cancer</td>
<td>ceritinib, crizotinib</td>
</tr>
<tr>
<td>BRAF mutation</td>
<td>Melanoma</td>
<td>dabrafenib, trametinib, vemurafenib</td>
</tr>
<tr>
<td>EGFR mutation</td>
<td>Non-Small Cell Lung Cancer</td>
<td>afatinib, erlotinib</td>
</tr>
<tr>
<td>ERBB2 amplification</td>
<td>Breast Cancer</td>
<td>pertuzumab, trastuzumab</td>
</tr>
<tr>
<td>ERBB2 amplification</td>
<td>Gastric Cancer</td>
<td>trastuzumab</td>
</tr>
<tr>
<td>KRAS mutation</td>
<td>Colorectal Cancer</td>
<td>cetuximab, panitumumab contraindicated</td>
</tr>
</tbody>
</table>

Hovelson et al., Neoplasia, 2015
Targeted Sequencing: Distinguishing Benign from Malignant Melanocytic Lesions

Somatic Mutations

NEVI

SPITZ NEVI

CONVENTIONAL MELANOMA

BRAF

RAS

NF1

No additional mutations

PPP6C, ARID2 etc
Targeted Sequencing: Resolving Mutational Timing in Melanomagenesis

Figure 4. Proposed Models for Progression of Melanomas Developing on Sun-Exposed Sites.

Shain et al. NEJM, 2015
Targeted Sequencing:
Drug Sensitivity and Resistance (RAC1 P29S)

NF1 → RAS → RAC1 → BRAF → MEK/ERK → PD-L1

BRAFi

P29S
Beyond Mutations: Fusion Genes in Triple WT Melanomas

- Triple WT Melanomas
  - No BRAF/RAS/NF1 mutation
  - ~15-20% of sun-exposed melanomas
  - Distinct amplification peak at the KDR/KIT locus (shared with sun-shielded melanomas)

- Exome-based Copy Number Analysis reveals kinase fusion proteins in triple WT melanomas

![Image of a graph showing BRAF expression]
Beyond Mutations: Fusion Genes in Triple WT Melanomas

PDE4DIP/BRAF
Beyond Mutations: Kinase Activation Through Alternative Transcription

Wiesner et al., Nature 2015

~10% Melanomas
Summary

- Massive melanoma exome sequencing reveals a new melanoma subtype with inactivating NF1 mutations

- NF1-mutations may be insufficient for full MAPK activation; need concurrent mutations in the MAPK pathway

- Evidence for NF1 co-mutations in RASopathy genes; mutations are documented disease causing and/or MAPK pathway activating

- The next step is the design of a targeted sequencing “array” with melanoma-specific genes aimed at patient treatment
Exome sequencing identifies recurrent mutations in NF1 and RASopathy genes in sun-exposed melanomas

Michael Krauthammer¹,², Yong Kong³, Antonella Bacchiocchi⁴, Perry Evans¹, Natapol Pornputtapong², Cen Wu⁵, James P McCusker², Shuangge Ma⁵, Elaine Cheng⁴, Robert Straub⁴, Merdan Serin⁴, Marcus Bosenberg²,⁴, Stephan Ariyan⁶, Deepak Narayan⁶, Mario Szoln⁷, Harriet M Kluger⁷, Shrikant Mane⁸,⁹, Joseph Schlessinger¹⁰, Richard P Lifton⁹,¹¹ & Ruth Halaban⁴
Acknowledgements

Yale Cancer Center/Dermatology
Ruth Halaban
Antonella Bacchiocchi
Elaine Cheng
Robert Straub

Surgeons/Clinician
Stephan Ariyan
Deepak Narayan
Mario Sznol
Harriet Kluger
Marcus Bosenberg

Genetics
Richard Lifton

Structure/Function Analysis
Joseph Schlessinger, Titus Boggon

Yale Center for Genome Analysis
Shrikant Mane

Bioinformatics/Biostatistics
Natapol Pornputtapong
Yong Kong
Jim McCusker
Ana Capatana
Shuangge Ma
Perry Evans
Cen Wu

Funding
NIH/NCI, Yale SPORE in Skin cancer
Melanoma Research Alliance
Gilead Sciences, Inc.
Dermatology Department
Yale Cancer Center
NF1 Mutations & Desmoplastic Melanomas

- A majority of desmoplastic melanomas (chronic sun exposed skin, older individuals, 4% of all melanomas) are NF1 mutants (50-90%).

- Recent publications highlights the occurrence of MAPK pathway mutations in desmoplastic melanomas (including PTPN11 mutations, SOS1 and others).

Shain et al., Nature Genetics, September 2015

PTPN11 p.76 (2x), p.461 (1x) (Noonan)
**NF1 and Concurrent MAPK Pathway Mutations**

![Pathway Diagram]

- **NF1**
- **RAS**
- **BRAF**
- **MEK**
- **ERK**

### Table

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>RASA (%)</th>
<th>DUSP (%)</th>
<th>SPRED/SPROUTY (%)</th>
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<tbody>
<tr>
<td><strong>BRAF</strong></td>
<td>94</td>
<td>11%</td>
<td>21%</td>
<td>3%</td>
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<tr>
<td><strong>RAS</strong></td>
<td>66</td>
<td>15%</td>
<td>24%</td>
<td>3%</td>
</tr>
<tr>
<td><strong>NF1</strong></td>
<td>27</td>
<td>48%</td>
<td>56%</td>
<td>19%</td>
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<td><strong>WT</strong></td>
<td>33</td>
<td>18%</td>
<td>18%</td>
<td>6%</td>
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Inactivating NF1 mutations do not necessarily lead to full RAS activation.

NF1 melanomas have increased mutation burden and concurrent MAPK pathway mutations (RASopathy genes).

**Is NF1 Loss is Insufficient for MAPK pathway activation?**

<table>
<thead>
<tr>
<th>Mean # of Mutations</th>
<th>Mean Age of Onset</th>
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<tr>
<td>0</td>
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<tr>
<td>466,6667</td>
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<td>933,3333</td>
<td>65</td>
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<td>1400</td>
<td>72.5</td>
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<td>7200</td>
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