In vivo genetic screens to discover regulators of tumor immunity

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Disclosures

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  – Third Rock Ventures
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  – Siamab Therapeutics

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  – Calico
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  – Roche
A brief history of tumor immunotherapy

1909
Bacterial toxins

2018
CAR-T cells
Checkpoint blockade
Engineer away T cell exhaustion
Increase response
Overcome resistance

Cure
Unmet need

CTLA-4 + PD-1 blockade in advanced melanoma

New IO targets

*in vivo* genetic screens

Adar1

Wolchok NEJM 2013
Mouse models of tumor immunity

**B16**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Melanoma</th>
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<tbody>
<tr>
<td>Derivation</td>
<td>Spontaneous</td>
</tr>
<tr>
<td>Immuno-genicity</td>
<td>Low</td>
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</tbody>
</table>

**GVAX**
GM-CSF secreting tumor vaccine

**PD-1 blockade**
Blocking ab to PD-1
Pooled loss-of-function screens for immunotherapy target discovery
Screen pipeline

Cas9-expressing cells

2d

MACS Purify

10d

2m cells

in vitro

TRCa ko

~14d

Harvest tumors and sequence

WT

GVAX

GVAX + anti-PD1

n=10/group

Manguso et al, Nature 2017
Titrated immune selective pressure
Curate list from relevant biological classes

Remove known essential genes

Remove genes not expressed by cell lines

2398 genes

4 gRNAs per gene

Pool 1  Pool 2  Pool 3  Pool 4

GO terms:
- Plasma membrane: 42.5%
- Kinase: 32%
- Phosphatase: 9.5%
- Cell surface: 8%
- Immune process: 4.5%
- Chromatin remodeling: 2%
- Antigen processing: 1.5%
Screen recovers known immune evasion molecules e.g. PD-L1

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Z-score</th>
<th>FDR</th>
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<tbody>
<tr>
<td>TCRα KO vs in vitro</td>
<td></td>
<td>ns</td>
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<tr>
<td>GVAX vs TCRα KO</td>
<td></td>
<td>0.004</td>
</tr>
<tr>
<td>GVAX + anti-PD-1 vs TCRα KO</td>
<td></td>
<td>ns</td>
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</table>

2,500 genes
10,000 sgRNAs
120 mice
1. TNF signaling/NF-κB activation

2. Antigen processing and presentation

3. Inhibition of kinase signaling

4. Ubiquitin proteasome pathway

5. Inhibition of dsRNA sensing

Multiple genes from diverse pathways sensitize tumors to immunotherapy
Sources of double stranded RNA

**Viruses**

**Our Genome**

- Exons (1.5%)
- Regulatory sequences (5%)
- Introns (~20%)
- Unique noncoding DNA (15%)
- Repetitive DNA that includes transposable elements and related sequences (44%)
- L1 sequences (17%)
- Alu elements (10%)
- Simple sequence DNA (3%)
- Large-segment duplications (5–6%)
Recognition of dsRNA triggers the anti-viral response
Adar prevents recognition of dsRNA

- **Adenosine Deaminase** that acts on RNA
- Catalyzes A-I editing in RNA
- Prevents sensing as invading viral RNAs
- ADAR1 knockout embryonic lethal due to upregulated interferon signaling
- Aicardi-Goutières Syndrome: ADAR1 mutation cause “Type I IFN interferonopathy” that mimics viral infection
Deletion of Adar does not affect cell viability *in vitro*
Adar loss profoundly sensitizes tumors to immunotherapy

B16 tumors

**NSG mice**

**WT mice**

**WT mice + anti-PD-1**

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**Percent survival**

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**Days after tumor challenge**

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Control sgRNA

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*Adar sgRNA (p150/p110)*

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*Adar sgRNA (p150)*

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anti-PD-1

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Increased T cell infiltrate in Adar1 null tumors
Loss of Adar1 reshapes the tumor microenvironment.

scRNA-seq of immune infiltrate in untreated B16 Tumor

Control sgRNA

Adar sgRNA
Increased expression of pro-inflammatory genes in Adar1 null tumors
Increased interferons in Adar null tumors

CD45+ cell gene expression

Cytokines in tumor lysate *ex vivo*

- **IFNβ**
  - Control sgRNA
  - Adar1 sgRNA

- **IFNγ**
  - Control sgRNA
  - Adar1 sgRNA

*Significance levels: *p* < 0.001*
Loss of Adar1 decreases dsRNA editing
A-I edits fall in short interspersed nuclear elements (SINEs) found in introns and 3’ UTRs.
Edited RNAs are SINEs are enriched in interferon-inducible genes

![Graph showing running enrichment score with FDR < 0.001 and 6679 regions containing A-I edits.](image)
Loss of Adar1 increases IFN secretion from tumor cells in response to IFN stimulation.

![Diagram showing the role of Adar1 in interferon secretion by tumor cells.](Image)

**Interferon secretion by tumor cells**

- **Type I Interferon**
- **Translational arrest**
- **Apoptosis**

**Legend for the chart:**
- **Control sgRNA**
- **Adar sgRNA (p150/p110)**
- **Adar sgRNA (p150)**

<table>
<thead>
<tr>
<th></th>
<th>IFNβ</th>
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<th>IFNγ</th>
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Loss of Adar decreases tumor cell growth in response to IFN stimulation.

Inhibition of growth:

- **MDA5** and **RIG-I** are downstream of dsRNA binding via Adar.
- PKR is activated by MDA5 and RIG-I.
- Type I Interferon leads to Translational arrest and Apoptosis.

Graph showing inhibition of growth with different treatments:

- Control sgRNA
- Adar sgRNA (p150/p110)
- Adar sgRNA (p150)

The graph plots cell count relative to unstimulated conditions with log2 fold change. The treatments include TNFα, IFNβ, and IFNγ.
Growth inhibition of Adar$^{null}$ tumors by IFN is dependent on PKR
Genetic epistasis of Adar phenotypes \textit{in vitro}

**In vitro**

<table>
<thead>
<tr>
<th>sgRNA 1</th>
<th>sgRNA 2</th>
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<tbody>
<tr>
<td>Control</td>
<td>Control</td>
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<tr>
<td>Adar1</td>
<td>Control</td>
</tr>
<tr>
<td>Adar1</td>
<td>PKR</td>
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<tr>
<td>Adar1</td>
<td>MAVS</td>
</tr>
<tr>
<td>Adar1</td>
<td>MDA5</td>
</tr>
<tr>
<td>Adar1</td>
<td>RIG-I</td>
</tr>
</tbody>
</table>

- **Growth Inhibition (IFNβ)**
- **Growth Inhibition (IFNγ)**
- **IFNβ Production**

**Bar Graphs**
- Growth Inhibition (IFNβ) with log2 fold change.
- Growth Inhibition (IFNγ) with log2 fold change.
- IFNβ Production with pg/ml.

**Legend**
- ADAR
- dsRNA
- MBA5
- RIG-I
- PKR
- Type I Interferon
- Translational arrest
- Apoptosis
Genetic epistasis of Adar sensitivity to PD-1 in vivo

- Growth inhibition (by PKR) or IFN production (by MDA5/MAVS) is sufficient to mediate sensitivity to immunotherapy
- Signaling through one or both is necessary
Loss of Functional Beta$_2$-Microglobulin in Metastatic Melanomas From Five Patients Receiving Immunotherapy

Nicholas P. Restifo, Francesco M. Marincola, Yutaka Kawakami, Jeff Taubenberger, John R. Yannelli, Steven A. Rosenberg*

J NCI 1996

Resistance to checkpoint blockade therapy through inactivation of antigen presentation

Moshe Sade-Feldman$^{1,2}$, Yunxin J. Jiao$^{2,3}$, Jonathan H. Chen$^{2,4}$, Michael S. Rooney$^{2}$, Michal Barzily-Rokni$^{1}$, Jean-Pierre Elane$^{6}$, Stacey L. Bjorgaard$^{1,2}$, Marc R. Hammond$^{1}$, Hans Vitzthum$^{1}$, Shauna M. Blackmon$^{1}$, Dennie T. Frederik$^{1}$, Mehlika Hazar-Rethinam$^{1}$, Brandon A. Nadres$^{1}$, Emily E. Van Seventer$^{1}$, Sachet A. Shukla$^{2,5}$, Keren Yizhak$^{5}$, John P. Ray$^{5}$, Daniel Rosebrock$^{2}$, Dimitri Livitz$^{2}$, Viktor Adalsteinsson$^{2}$, Gad Getz$^{2,6}$, Lyn M. Duncan$^{7}$, Bo Li$^{6}$, Ryan B. Corcoran$^{1}$, Donald P. Lawrence$^{1}$, Anat Stemmer-Rachamimov$^{2}$, Genevieve M. Boland$^{7}$, Dan A. Landau$^{2,8,9}$, Keith T. Flaherty$^{1}$, Ryan J. Sullivan$^{1}$ & Nir Hacohen$^{2}$

Nat Comm 2017
Modeling resistance from B2M loss

Events

H2K(b)-H2D(b)

Control sgRNA
B2m sgRNA
Isotype control

OT-I or TCRαko CD8 T cells

Wild-type
B2M null
+/- ovalbumin

Depleted Enriched relative to no T cell control (log₂ fold change)

E.T Ratio

OVA- B16
OVA+ Control sgRNA
OVA+ B2m sgRNA
Adar deletion resensitizes resistant tumors to immunotherapy
Adar deficient tumors still recruit effector populations even in the absence of CD8+-mediated recognition of tumor cells
Adar functions as a checkpoint that limits dsRNA sensing in tumors

- Adar inhibition potentiates immunotherapies and can overcome resistance by enhancing inflammation and reshaping the tumor microenvironment.
- Consequences of Adar deletion suggest that sufficient innate ligand exists in tumor cells to elicit inflammation if sensing checkpoint is removed.
- Inducing sufficient inflammation in tumors that are sensitized to interferon can bypass the therapeutic requirement for CD8+ T cell recognition of cancer cells.
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