Comprehensive Pan-Genomic Characterization of Adrenocortical Carcinoma

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Update on behalf of the ACC AWG

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Adrenocortical Carcinoma

- Rare; 1-2 cases per million
  - 500 cases in US per year
- Variable outcome, dependent on grade and stage
- Associated endocrinopathies
  - e.g. hypercortisolism (Cushing syndrome)
- Limited therapeutic options
- Centers with multidisciplinary clinics
  - Michigan, MD Anderson, NIH
Adrenal Cortical Carcinoma (ACC)

Very large - 20 cm tumor
Adenoma to Carcinoma Progression
Resection - Only Curative Treatment
Stage

• Stage I
  – Less than 5 cm and confined to the adrenal
  – Rare cases
• Stage II
  – Greater than 5 cm and confined to the adrenal
• Stage III
  – Any size; locally invasive
• Stage IV
  – Any size; distant metastatic disease
Grade:
Two Mitotic Grades

Low: less than 20 mitoses/50 hpfs
High: 20 or more mitoses/50 hpfs
Range of Morphologies
Intratumoral Heterogeneity
Challenges

• Diagnostically difficult intermediate cases
• Overall prognosis assessment
• Prediction and risk assessment
  – Risk of local recurrence
  – Risk of metastatic disease
  – Response to therapy
• Limited therapeutic options
### Emerging Molecular Classification

Transcriptome-based tumour classification

<table>
<thead>
<tr>
<th>Molecular events</th>
<th>Carcinomas (ACC)</th>
<th>Adenomas (ACA)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gene expression</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>IGF2</em> overexpression</td>
<td>Poor prognosis</td>
<td>Good prognosis</td>
</tr>
<tr>
<td>Overexpression of cell-cycle-related genes</td>
<td></td>
<td></td>
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<tr>
<td>Overexpression of steroidogenic genes</td>
<td></td>
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<tr>
<td><strong>Chromosomal alterations</strong></td>
<td>High number of chromosomal alterations</td>
<td></td>
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<tr>
<td></td>
<td>Poor prognosis</td>
<td>Good prognosis</td>
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<tr>
<td><strong>miRNA expression</strong></td>
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<tr>
<td>High miR-483 expression</td>
<td>Poor prognosis</td>
<td>Good prognosis</td>
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<tr>
<td>High miR-195 expression</td>
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<tr>
<td><strong>DNA methylation</strong></td>
<td></td>
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<tr>
<td>Normal methylation in intergenic regions</td>
<td>Poor prognosis</td>
<td>Good prognosis</td>
</tr>
<tr>
<td>Hypermethylation of CpG island in gene promoter regions</td>
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<tr>
<td><strong>Other features</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High mitotic grade</td>
<td>Poor prognosis</td>
<td>Good prognosis</td>
</tr>
<tr>
<td>p53 alteration</td>
<td>Poor prognosis</td>
<td>Good prognosis</td>
</tr>
<tr>
<td>Wnt/β-catenin activation</td>
<td>Poor prognosis</td>
<td>Good prognosis</td>
</tr>
</tbody>
</table>

- **The feature is strongly present**
- **The feature is moderately present**
- **The feature is absent**
Profiling Results in Agreement with Mitotic Grade
TCGA ACC Cohort & Data

- Global cohort; NA, SA, EU, & AUS
- Whole exome sequencing, n = 91
- mRNA sequencing, n=78
- miRNA sequencing, n=79
- DNA copy number, n=89
- DNA methylation, n=79
- RPPA, n=45
- Clinical data
- Pathology data
ACC Mutation density
Mutation Density Correlates, all stages
Mutation Density Correlates, stage I and II only

<table>
<thead>
<tr>
<th>Mutation Load</th>
<th>High</th>
<th>Intermediate</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
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<td></td>
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<tr>
<td>Stage</td>
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<td></td>
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<tr>
<td>Weiss score</td>
<td></td>
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<td></td>
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<tr>
<td>Vital status</td>
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<td></td>
<td></td>
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<tr>
<td>Progression</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Necrosis</td>
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<td></td>
<td></td>
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<tr>
<td>Mitotic rate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitoses count</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cluster of clusters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copy number</td>
<td></td>
<td></td>
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<tr>
<td>Genome doubling</td>
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</tbody>
</table>

* p-values for each correlation:
  - High to Low: p=0.01
  - Intermediate to Low: p=0.39
  - Weiss score: p=0.0018
  - Vital status: p=0.038
  - Progression: p=0.012
  - Necrosis: p=0.045
  - Mitotic rate: p=0.031
  - Mitoses count: p=0.0085
  - Cluster of clusters: p=0.00016
  - Expression: p=0.0052
  - Methylation: p=9.7e-06
  - Copy number: p=4.3e-05
  - Genome doubling: p=0.0075
Whole exome sequencing:
Significantly mutated genes using MutSigCV
Hunt for Gene Fusions

• Excited about the prospect of finding recurrent gene fusions
• Used two methods to detect fusions
• Found 156 fusion events in 48 of 78 tumors
• Copy number data indicated a breakpoint in 65% of cases
No Recurrent Fusions
Some Private Fusions involve known Cancer Genes
Copy Number Alterations circa 2000

[Diagram of chromosomes and copy number alterations]

Genes, Chromosomes and Cancer
Focal copy number alterations

Novel TERF2 amplification

Maybe unique within TCGA
**TERF2/TRF2 –**
Telomeric Repeat Binding Protein 2

*Nature* **447**, 924-931
ZNRF3 deletion

- Negative regulator of Wnt signaling pathway
- Leads to degradation of Wnt receptor complex proteins
- Deletion of ZNRF3 may represent an alternative way to activate Wnt pathway in ACC
- About 20% of ACCs
Copy number defines different classes of ACC...

Roel Verhaak, Siyuan Zhang, Andy Cherniack, Brad Murray, others
With different survival

Noisy tumors most aggressive

$P \leq 10^{-3}$, log-rank test
PanCancer Context, purity and ploidy

Graph C: Purity and ploidy distribution for different cancer types.

- **Purity** distribution across various cancer types.
- **Ploidy** distribution with different colors representing different ploidy levels (red for 2, green for 1, blue for 0).

Cancer types and their respective purity and ploidy values:

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Purity</th>
<th>Ploidy</th>
</tr>
</thead>
<tbody>
<tr>
<td>LUAD</td>
<td>58.6</td>
<td>1.7</td>
</tr>
<tr>
<td>LUSC</td>
<td>64</td>
<td>1.1</td>
</tr>
<tr>
<td>HNSC</td>
<td>43</td>
<td>0</td>
</tr>
<tr>
<td>KIRC</td>
<td>20.1</td>
<td>1.3</td>
</tr>
<tr>
<td>BRCA</td>
<td>45.4</td>
<td>1.5</td>
</tr>
<tr>
<td>BLCA</td>
<td>62.2</td>
<td>0</td>
</tr>
<tr>
<td>CRC</td>
<td>43.1</td>
<td>0</td>
</tr>
<tr>
<td>THCA</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>UCEC</td>
<td>27.1</td>
<td>0.5</td>
</tr>
<tr>
<td>GBM</td>
<td>10.7</td>
<td>0.8</td>
</tr>
<tr>
<td>OV</td>
<td>53.4</td>
<td>0.2</td>
</tr>
<tr>
<td>ACC</td>
<td>51.2</td>
<td>31</td>
</tr>
<tr>
<td>KICH</td>
<td>14.5</td>
<td>37.1</td>
</tr>
</tbody>
</table>

Legend:
- **WGD**: Whole-genome duplication
- **% WGD**: Percentage of whole-genome duplication
- **% Hyper**: Percentage of hyperdiploidy
Transcriptome

2 large classes

4 granular classes

profound differences between classes

Richard Moffett, others
Methylation
Methylation validation

68-probe methylation signature
Most *CTNNB1* mutations in Groups II and III
Supervised Landscape View
Integrative Analyses

- Telomere length
- Adrenocortical differentiation
- HotNets
- PHIAL
- OncoSign
- PanCancer oncogenic processes
Telomere Length – ALT in play

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**Telomere Length**

- TERT Expression
- TERT mut/amp
- TERF2 amp
- Telomere length
- LoG2(Tumor/Normal)
- ATRX/DAXX mut
- MLL mut

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- N=61 (73%)
- N=23 (27%)
Pan-cancer mutation signature analysis
Highlights

• Created a outstanding genomic resource for adrenal cancer research
• Discovered novel somatic alterations
• Expanded role of WNT pathway alterations
• Copy number / whole genome doubling
• COC analysis with integrated view
  – Overall 3 classes of tumors
• Pan-cancer analysis
• Paper under review
Looking forward

• Pathology
  – Looking for ways to deliver the 3 class solution to routine cases

• Therapy
  – Supports the view that combined inhibition of IGF2 and Wnt pathways for the largest subset of cases
  – Other pathways in smaller subsets
People

Roel Verhaak

Siyuan Zheng
Many More People


- Plus many more (TSSs, etc.) from TCGA network
- TCGA program office
- **Kenna Shaw for listening and supporting the project**