Molecular Subtypes of Renal Cell Carcinoma

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• No disclosures
Learning Objectives

• Familiarization with the genomic landscape of Renal Cell Carcinoma
• Integrative approach to Molecular Subtyping of RCCs
• Challenges to molecular classification of RCCs
Outline

• Introduction
• Treatment strategies
• Genomic Landscape of RCC
  • Histopathological and molecular subtypes
  • Genomic correlates with clinical outcomes
  • Integrated Multi-omics across RCC subtypes
• Immunotherapy Biomarkers
• Challenges to Molecular Classification of RCCs
• Conclusion
# Renal Cell Carcinomas: Subtypes

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Percentage</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear cell RCC</td>
<td>75%</td>
<td>Medullary RCC</td>
</tr>
<tr>
<td>Papillary RCC</td>
<td>15%</td>
<td>Collecting duct carcinoma</td>
</tr>
<tr>
<td>Chromophobe RCC</td>
<td>5%</td>
<td>MiTF-RCC</td>
</tr>
<tr>
<td>Clear cell papillary RCC</td>
<td>4%</td>
<td>FH deficient RCC and/or HLRCC</td>
</tr>
<tr>
<td>Unclassified RCC</td>
<td>4%</td>
<td>SDH deficient RCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tubulocystic RCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multilocular cystic renal neoplasm of low malignant potential</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mucinous tubular and spindle cell carcinoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acquired cystic disease-associated RCC</td>
</tr>
</tbody>
</table>

• About 30% of patients present with metastatic disease at the time of diagnosis
• An additional 30% of patients with localized RCC, despite surgery with curative intent, eventually develop recurrence or metastasis
RCC: Treatment Strategies
NCCN Guidelines

- Determined by
  - Tumor Stage
  - Amenability to resection
  - Co-morbidities
- Systemic Therapy: Surgically unresectable/advanced disease/ metastatic disease
### Targeted therapies approved for RCC

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGFR inhibitors</td>
<td>Sunitinib, Pazopanib, Bavacizumab</td>
</tr>
<tr>
<td>mTORC1 inhibitors</td>
<td>Temsorilimus, Everolimus</td>
</tr>
<tr>
<td>C-MET inhibitors</td>
<td>Cabozantinib</td>
</tr>
<tr>
<td>FGFR inhibitors</td>
<td></td>
</tr>
<tr>
<td>Cytokines</td>
<td>Interluekin-2, Interferon-α</td>
</tr>
<tr>
<td>Anti-PD1/PD-L1</td>
<td>Nivolumab</td>
</tr>
</tbody>
</table>

- **Other targetable pathways/ alterations:**
  - Hippo
  - NRF2-ARE
  - MAP kinase
  - ALK
  - CHECK2/PBRM1
  - ATM/BRCA2

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Genomic Landscape of RCC
### TABLE 1. Hereditary RCC Syndromes

<table>
<thead>
<tr>
<th>Syndromes</th>
<th>Gene</th>
<th>Histologic Types of Renal Tumors</th>
<th>Incidence of Renal Cancer and Mean Age at Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>VHL disease</td>
<td>VHL 3p25-26</td>
<td>Clear cell RCC</td>
<td>25%-45% 40 y</td>
</tr>
<tr>
<td>Hereditary papillary RCC</td>
<td>MET 7q31</td>
<td>Papillary RCC type 1</td>
<td>Unknown &lt; 60 y</td>
</tr>
<tr>
<td>BHD syndrome</td>
<td>BHD 17p11.2</td>
<td>Hybrid oncocytic/chromophobe RCC Oncocytoma Clear cell RCC Papillary RCC</td>
<td>34% 50 y</td>
</tr>
<tr>
<td>HLRCC</td>
<td>FH 1q42-43</td>
<td>Heterogenous, but predominantly papillary RCC type 2-like</td>
<td>2%-21% 46 y</td>
</tr>
<tr>
<td>TSC</td>
<td>TSC1/TSC2 9q34/16p13</td>
<td>AML Renal cysts Papillary RCC Clear cell RCC Oncocytoma</td>
<td>2%-4% 30 y</td>
</tr>
<tr>
<td>Hereditary paraganglioma-pheochromocytoma syndrome</td>
<td>SDHB/SDHC/SDHD 1p36/1q21/11q23</td>
<td>Clear cell RCC</td>
<td>5%-15% 30 y</td>
</tr>
<tr>
<td>Hereditary sickle cell hemoglobinopathy and medullary RCC</td>
<td></td>
<td>Medullary RCC</td>
<td>10-30 y</td>
</tr>
<tr>
<td>Germline PTEN mutation Cowden syndrome</td>
<td>PTEN 10q22-23</td>
<td>Clear cell RCC Papillary RCC Chromophobe RCC</td>
<td>34% 40 y</td>
</tr>
<tr>
<td>Hyperparathyroidism-jaw tumor syndrome</td>
<td>HRPT2 1q21-32</td>
<td>Mixed epithelial and stromal tumor Papillary RCC Wilms tumor</td>
<td>—</td>
</tr>
<tr>
<td>BAP1 mutations and familial kidney cancer</td>
<td>BAP1 3p21</td>
<td>Clear cell RCC</td>
<td>—</td>
</tr>
<tr>
<td>Constitutional chromosome 3 translocation RCC</td>
<td>Unknown chromosome 3</td>
<td>Clear cell RCC</td>
<td>Unknown</td>
</tr>
</tbody>
</table>
**TCGA Pan-Kidney Cancer Analysis (n=843)**

**Clear Cell RCC**
- Increased ribose metabolism pathway mRNA expression associated with poor survival
- Increased immune signature

**Chromophobe RCC**
- Identification of metabolically divergent (MD-) ChRCCs associated with extremely poor survival

**Type 1 Papillary RCC**
- *PBRM1* mutations associate with poor survival
- Increased mRNA signature for RNA splicing and cilia genes

**Type 2 Papillary RCC**
- Increased expression of the glycolysis, ribose metabolism, and Krebs cycle genes in comparison to Type 1 PRCC

**Renal Cell Carcinoma (RCC)**
- Increased DNA hypermethylation and CDKN2A alterations associate with poor prognosis in all RCC subtypes
- Increased Th2 immune signature within each RCC subtype associates with poor survival
4 Classification Categories

- Histopathology
- Molecular Pathology
- Genomic correlates with clinical outcomes
- Integrated Multi-omics across RCC subtypes

Histopathology and Molecular Pathology
Clear Cell RCC

- Majority- sporadic
- <5%- inherited cancer syndromes
Clear Cell RCC

- VHL/ 3p LOH (90%)
- Deletion of 3p >90% (biallelic)- 3 genes
  - VHL: Tumor suppressor
  - PBRM1- chromatin remodeling complex
  - BAP1, SETD2, JARID1
- Epigenetic silencing in ~7%, mutually exclusive with mutation
- Inactivation of VHL serves as the fundamental driver event of human ccRCC

ROS

Prolyl hydroxylase, 2OG

E3 ubiquitin ligase complex

HIF 1-α

Anaerobic Glycolysis

VEGF EPO CAIX
### Common Genetic Alterations in ccRCCs

<table>
<thead>
<tr>
<th>Mutations in 93% of ccRCC</th>
<th>Percentage of cases</th>
<th>Clinical Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>VHL</td>
<td>&gt;70%</td>
<td>Diagnostic</td>
</tr>
<tr>
<td>PBRM1</td>
<td>~ 40%</td>
<td>Longer survival on MTORI</td>
</tr>
<tr>
<td>BAP1</td>
<td>~ 15-20%</td>
<td>High grade, poor outcomes on VEGFR TKI/ MTOR Inhibitor</td>
</tr>
<tr>
<td>SETD2</td>
<td>~ 7-11%</td>
<td>Worse survival, associated with metastases</td>
</tr>
<tr>
<td>KDM5C</td>
<td>~ 14%</td>
<td>Longer survival on VEGF TKI</td>
</tr>
<tr>
<td>TP53</td>
<td>2.2 – 8%</td>
<td>High grade, decreased survival</td>
</tr>
<tr>
<td>PIK3CA</td>
<td></td>
<td>Targetable</td>
</tr>
<tr>
<td>MTOR</td>
<td>~ 5%</td>
<td>Response to MTORI, mutations in metastases better response than mutations in primary</td>
</tr>
<tr>
<td>TSC1</td>
<td></td>
<td>Targetable</td>
</tr>
<tr>
<td>NF2</td>
<td>~ 3%</td>
<td>Targetable</td>
</tr>
</tbody>
</table>


Copy Number Changes: ccRCCs

Papillary RCC

- Gain of chromosomes 7 and 17
- Loss of Y chromosome
- Hereditary pRCC
  - c-Met gene mutations, AD
  - No extra renal manifestations
  - Bilateral, multiple, multifocal type 1 pRCCs/adenomas
- Sporadic Type 1 pRCC- MET gene mutations (13%)
  - MET inhibitors
- Type 2 pRCC- Heterogeneous group
Papillary RCCs

- Type 1 pRCC: MET (trisomy 7): Targetable with MET/VEGFR2 inhibitors
- Type 2 pRCC
  - CDKN2A silencing (Chr 9p21 loss); decreased overall survival
  - SETD2 mutations
  - TFE3 fusions
  - NRF2-ARE (antioxidant response element) pathway (increased expression)
    - CUL3 mutations
    - NRF2 mutations
- NF2 mutations: Targetable by YES1 kinase inhibitors (Dasatinib)
- TERT promoter mutations

A Distinct pRCC Subtype

- CpG Island Methylator Phenotype
  - Universal hypermethylation of CDKN2A promoter
  - 5.6% of papillary RCCs
  - FH mutations ~ 56%
  - Earlier age of presentation
  - Decreased survival
  - Warburg like metabolic shift

Molecular Differences Between Type 1 & 2 pRCCs

<table>
<thead>
<tr>
<th>Genes/Pathways</th>
<th>Type 1 (%)</th>
<th>Type 2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF2</td>
<td>2.8%</td>
<td>10.0%</td>
</tr>
<tr>
<td>Hippo signaling pathway</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMARCB1, PBRM1</td>
<td>19.7%</td>
<td>26.7%</td>
</tr>
<tr>
<td>SWI/SNF complex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SETD2, KDM6A, BAP1</td>
<td>35.2%</td>
<td>38.3%</td>
</tr>
<tr>
<td>Chromatin remodeling pathways</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Copy Number Changes: pRCCs

Genome stable, sporadic SCNAs, few Chr 9p deletions

Arm-level alterations, Chr 7 gain

Genome unstable, Chr 9p deleted
Chromophobe RCC

- Multiple complex chromosomal losses (Hypodiploid)
  - 1, 2, 6, 10, 13, 17 and 21 (7-set)
- TERT promoter (10%)
- TP53 (32%)
- PTEN (9%)
- Mitochondrial DNA mutations

Aggressive Chromophobe RCCs

- Metastatic ChRCC: ~10-15%
- Casuscelli et al
  - Integrated analyses of 79 chRCC patients, 38 with metastatic disease
    - Whole-genome sequencing
    - Targeted exome sequencing
    - OncoScan
    - FACETS
    - FISH
  - High-risk genomic features: Any of the 3
    - TP53 mutation
    - PTEN mutation
    - Imbalanced chromosome duplication

Aggressive Chromophobe RCCs

Unclassified RCC

- 4-5%
- Adverse histological features, heterogeneous
- Aggressive biological potential
- Higher rate of nodal and/or distant metastases at presentation
- Low survival rates
### Aggressive Unclassified RCCs

<table>
<thead>
<tr>
<th>55%</th>
<th>45%</th>
</tr>
</thead>
<tbody>
<tr>
<td>• NF2 loss and dysregulated Hippo–YAP signaling (18%)</td>
<td>• Chromatin modulation (13%)</td>
</tr>
<tr>
<td>• Worse outcomes</td>
<td>• Intermediate outcomes</td>
</tr>
<tr>
<td>• Hyperactive mTORC1 signaling (26%)</td>
<td>• (SETD2, BAP1, KMT2A/C/D, PBRM1)</td>
</tr>
<tr>
<td>• Better outcomes, therapeutic target</td>
<td>• DNA damage response (8%)</td>
</tr>
<tr>
<td>• MTOR, TSC1, TSC2, PTEN</td>
<td>• (TP53, CHEK2, BRCA2)</td>
</tr>
<tr>
<td>• FH: worse outcomes</td>
<td>• No recurrent molecular features (24%)</td>
</tr>
<tr>
<td>• ALK</td>
<td></td>
</tr>
</tbody>
</table>

## Other RCC Subtypes

<table>
<thead>
<tr>
<th>RCC Subtype</th>
<th>Molecular Alterations</th>
</tr>
</thead>
</table>
| Collecting Duct Carcinoma | NF2 (5/17)  
SETD2 (4/17)  
SMARCB1 (3/17)  
FH (2/17)  
CDKN2A (2/17) |
| Medullary RCC           | SMARCB1/INI: LOH/ balanced translocations/ biallelic loss                           |
| TFE3 RCC                | Translocations with SFPQ, ASPSCR1, PRCC, NONO, CLTC, KSHRP, and LUC7L3              |
| Sarcomatoid RCCs        | TP53, BAP1, ARID1A, PTEN, CDKN2A, and NF2                                          |
Genomic correlates with clinical outcomes
DNA methylation patterns

- 10 subtypes
  - KIRC + KIRP (type 2): hypermethylation and poor outcomes
  - 4 subtypes of KIRC, 2 of which were enriched for BAP1 and associated with poor outcomes
  - 2 subtypes of KIRP

<table>
<thead>
<tr>
<th>KIRP</th>
<th>Morphological pattern</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 1</td>
<td>Type 1, MET mutation, Chr 7+</td>
<td>Low tumor stage. Best survival</td>
</tr>
<tr>
<td>Cluster 2a</td>
<td>Type 2</td>
<td>Low tumor stage, Best survival</td>
</tr>
<tr>
<td>Cluster 2b</td>
<td>Type 2, unclassified papillary RCC,</td>
<td>High tumor stage. Poor survival</td>
</tr>
<tr>
<td>Cluster 2c</td>
<td>CIMP tumor subtype NRF2-ARE pathway alterations</td>
<td>Worst survival</td>
</tr>
</tbody>
</table>

DNA Methylation

miRNA: CCRCC

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-21</td>
<td>worse outcomes, role in metabolism</td>
</tr>
<tr>
<td>miR-21, miR-10b, miR-30a</td>
<td>inversely correlated with DNA promoter methylation</td>
</tr>
<tr>
<td></td>
<td>Significant component of epigenetic regulation</td>
</tr>
</tbody>
</table>
miRNA: ccRCC

Metabolomic classification ccRCC

- mCluster 1-4
  - mCluster 2: High glutathione, worse outcomes
  - mCluster 3: High dipeptides, worse outcomes
  - mCluster 4: Low glutathione, better outcomes
  - mCluster 1: Low dipeptides, better outcomes
Metabolomic classification

- Up regulation of oxidative phosphorylation genes: Ch-e
- Down regulation of oxidative phosphorylation genes
  - ccRCC, P.CIMP-e
  - MAP kinase: ccRCC
  - NRF2-ARE (antioxidant response element), HIPPO pathways: P.CIMP-e
  - Loss of NF2: P.CIMP-e
  - PI3K/AKT/mTOR: ccRCC, pRCC

- Worse survival:
  - Pentose phosphate pathway
  - Fatty acid synthesis
  - PI3K pathway genes

- Better survival
  - AMPK
  - Krebs cycle
  - PI3K pathway inhibitor genes

Integrated Multi-omics across RCC subtypes
# 9 Molecular Subtypes

<table>
<thead>
<tr>
<th>RCC Subtype</th>
<th>Molecular Subtype</th>
<th>Molecular and Clinical Correlates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear cell</td>
<td>m1</td>
<td>Chromatin remodeling gene alterations, PBRM1 mutations: ccA</td>
</tr>
<tr>
<td></td>
<td>m2</td>
<td>ccB</td>
</tr>
<tr>
<td></td>
<td>m3</td>
<td>CDKN2A deletions, PTEN mutations: ccB</td>
</tr>
<tr>
<td></td>
<td>m4</td>
<td>BAP1 and mTOR mutations</td>
</tr>
<tr>
<td>Papillary Type 1</td>
<td>P-e.1a</td>
<td>Better</td>
</tr>
<tr>
<td></td>
<td>P-e.1b</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Papillary Type 2</td>
<td>P-e.2</td>
<td>Hypermethylation; intermediate; included cases with TFE3 fusions</td>
</tr>
<tr>
<td></td>
<td>P-CIMP-e</td>
<td>Hypermethylation; enriched for hereditary pRCC, CDKN2A loss/silencing, FH</td>
</tr>
<tr>
<td>Chromophobe</td>
<td>Ch-e</td>
<td></td>
</tr>
</tbody>
</table>
Immunotherapy Biomarkers
PD-L1/PD-1 Inhibitors

- ccRCC: High expression of several immunotherapy gene targets
  - Greater levels of immune infiltrates
- Many poor risk and Sarcomatoid tumors
  - High levels of PD-L1 expression
  - Greatest relative benefit with nivolumab over everolimus
- CheckMate 025 trial: Higher PD-L1 expression
  - Poor survival
  - No correlation with increased response rate to Nivolumab

PD-1/PD-L1 Challenges

- Different antibodies
- Immune infiltrating cells evaluated
- Intratumoral and intertumoral heterogeneity of PD-L1 expression
- Temporal evolution of PD-L1 status during the development of treatment resistance
- Variation in PD-L1 expression according to the level of tissue hypoxia
Mutational Load

RCC: Low mutational burden

• Highest number of small insertions and deletions of all cancer types
• Insertions/deletions: result in 3 times more immunogenic high-binding affinity neoantigens
• Microsatellite instability, BRCA1: targetable

Challenges to Molecular Classification of RCCs

- Marked intra and inter-tumoral heterogeneity
- Mutations different between primary and metastatic tumors
- Most genes are tumor suppressors with loss of function, not directly targetable
- Methylation, copy number loss, miRNA: not detectable by DNA mutation platforms
- Bionikk (phase 2BIOmarker driven trial)
  - Molecular classification
  - Nivolumab plus ipilimumab/ Nivolumab
  - Nivolumab plus ipilimumab/ TKI
Conclusion

• Integrated multi-omics approach
  • Molecular subtypes of RCCs

• Ongoing research
  • To improve therapeutic approach to RCCs
  • Identify biomarkers relevant to therapy
  • Research into RCC subtypes
Thank you