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
Renal Cell Carcinomas: Subtypes

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


RCC: Prognosis

- 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

- VEGFR inhibitors: Sunitinib, Pazopanib, Bavacizumab
- mTORC1 inhibitors: Temsirolimus, Everolimus
- C-MET inhibitors: Cabozantinib
- FGFR inhibitors: Nivolumab
- Cytokines: Interleukin-2, Interferon-α
- Anti-POI/PD-L1: Nivolumab

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

Genomic Landscape of RCC

Hereditary RCC Syndromes

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Type</th>
<th>Incidence of Renal Cancer and Risk of Renal Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>VHL disease</td>
<td>PHE.25/26</td>
<td>Clear cell RCC: 3%–10%</td>
</tr>
<tr>
<td>Hereditary papillary RCC</td>
<td>ARV.25/26</td>
<td>Papillary RCC type 1: 8%–16%</td>
</tr>
<tr>
<td>BHD syndrome</td>
<td>ARV.25/3</td>
<td>Renal oncocytoma/chromophobe RCC: 4%–8%</td>
</tr>
<tr>
<td>NS-VHL</td>
<td>PHE.3/2</td>
<td>Renal oncocytoma/renal cell RCC: 7%–12%</td>
</tr>
<tr>
<td>TSC</td>
<td>TSC.3/4</td>
<td>Hilar RCC: 5%–10%</td>
</tr>
</tbody>
</table>


TCGA Pan-Kidney Cancer Analysis (n=843)

- Clear Cell RCC: Increased HIF-1a-HIF2a-VEGFA pathway and HIF1A expression associated with poor survival.
- Chromophobe RCC: Identification of fibroblast growth factor receptor 3 (FGFR3) mutations and IDH1-R132C mutations.
- Type 1 Papillary RCC: Increased expression of the glycolytic enzymes hexokinase and fructose-1,6-bisphosphatase.
- Type 2 Papillary RCC: Increased expression of the glycolytic enzymes pyruvate kinase M2 and lactate dehydrogenase A.

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
- Histone modifying and chromatin remodeling genes: major roles for epigenetic regulation
Clear Cell

- 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


<table>
<thead>
<tr>
<th>Gene</th>
<th>Percentage of cases</th>
<th>Prognostic/therapeutic impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>VHL</td>
<td>&gt;70%</td>
<td>Diagnostic, no prognostic impact</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 metastasis</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>TSC1</td>
<td>~3%</td>
<td>Targetable</td>
</tr>
<tr>
<td>NF2</td>
<td></td>
<td>Targetable</td>
</tr>
</tbody>
</table>


The image contains a diagram illustrating the genetic basis of kidney cancer, with specific genes and their associated outcomes detailed in the table.
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 pRCC/adenomas
- Sporadic Type 1 pRCC: MET gene mutations (13%)
  - MET inhibitors
- Type 2 pRCC: Heterogeneous group
pRCC

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


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
    - Oncologic
    - FACETS
    - FISH
  - High-risk genomic features: Any of the 3
    - TP53 mutation
    - PTEN mutation
    - Imbalanced chromosome duplication


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

55%

- NF2 loss and dysregulated Hippo–YAP signaling (18%)
- Worse outcomes
- Hyperactive mTORC1 signaling (26%)
- MTOR, TSC1, TSC2, PTEN
- FH: worse outcomes
- ALK

45%

- Chromatin modulation (13%)
- Intermediate outcomes
- (SETD2, BAP1, KMT2A/C/D, PBRM1)
- DNA damage response (8%)
- (TP53, CHEK2, BRCA2)
- No recurrent molecular features (24%)


Other RCC Subtypes

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Medullary RCC</td>
<td>SMARC3/IM: LOH/ balanced translocations/ isochromosomal</td>
</tr>
<tr>
<td>TFE3 RCC</td>
<td>Translocations with SFPQ, ASPSCR1, PRCC, NONO, CTIC, KSHPR, and LUC7L3</td>
</tr>
<tr>
<td>Sarcomatoid RCCs</td>
<td>TP53, BAP1, ARID1A, PTEN, CDKN2A, and NF2</td>
</tr>
</tbody>
</table>


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>Subtype</th>
<th>Morphological pattern</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 1</td>
<td>Type 1, MET mutation, Chr7+</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>Type2, undifferentiated 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>

miRNA: CCRCC

- miR-21: worse outcomes, role in metabolism
- miR-21, miR-10b, miR-30a: inversely correlated with DNA promoter methylation
- Significant component of epigenetic regulation
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

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