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

		<1%
Clear cell RCC	75%	Medullary RCC
Papillary RCC	15%	Collecting duct carcinoma
Chromophobe RCC	5%	MiTF-RCC
Clear cell papillary RCC	4%	FH deficient RCC and/or HLRCC
Unclassified RCC	4%	SDH deficient RCC
		Tubulocystic RCC
		Multilocular cystic renal neoplasm of low malignant potential
		Mucinous tubular and spindle cell carcinoma
		Acquired cystic disease-associated RCC

Hseieh JJ et al. Genomic classifications of renal cell carcinoma: a critical step towards the future application of personalized kidney cancer care with pan-omics precision. J Pathol 2018; 244: 525–537

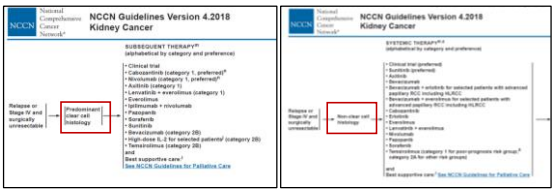
RCC: Prognosis

- 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

Hseieh JJ et al. Genomic classifications of renal cell carcinoma: a critical step towards the future application of personalized kidney cancer care with pan-omics precision. J Pathol 2018; 244: 525–537

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	Temsorilimus, Everolimus
C-MET inhibitors	Cabozantinib
FGFR inhibitors	
Cytokines	Interluekin-2, Interferon-α
Anti-PD1/PD-L1	Nivolumab

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

Hseieh JJ et al. Genomic classifications of renal cell carcinoma: a critical step towards the future application of personalized kidney cancer care with pan-omics precision. J Pathol 2018; 244: 525–537

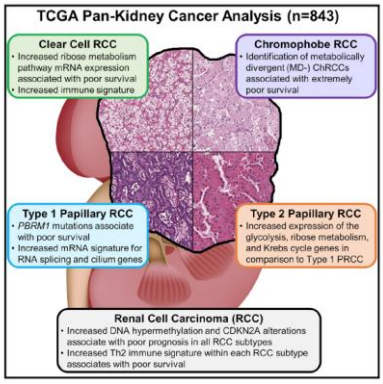
Genomic Landscape of RCC

Hereditary RCC Syndromes

TABLE 1. Hereditary RCC Syndromes

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

Adeniran AJ et al. Hereditary Renal Cell Carcinoma Syndromes: Clinical, Pathologic, and Genetic Features. Am J Surg Pathol 2015;39(12): e1-e18



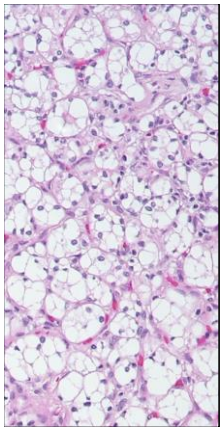
Ricketts et al. The Cancer Genome Atlas Comprehensive Molecular Characterization of Renal Cell Carcinoma. Cell Reports 2018;23:313–326

4 Classification Categories

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

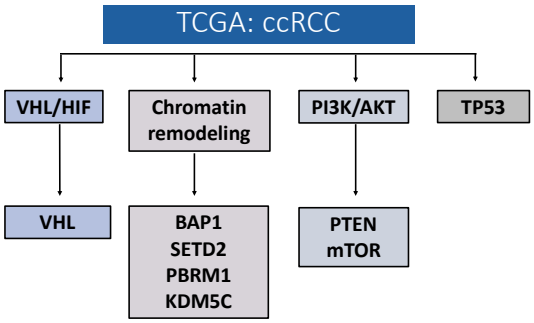
Hseieh JJ et al. Genomic classifications of renal cell carcinoma: a critical step towards the future application of personalized kidney cancer care with pan-omics precision. J Pathol 2018; 244: 525–537

Histopathology and Molecular Pathology



Clear Cell RCC

- Majority- sporadic
- <5%- inherited cancer syndromes

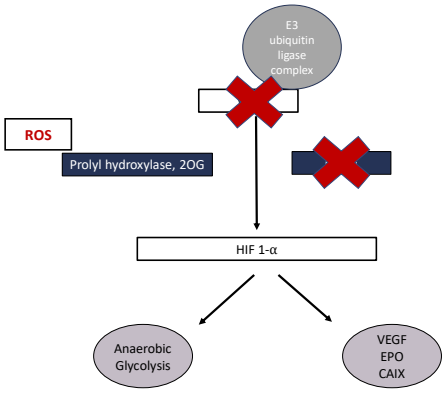


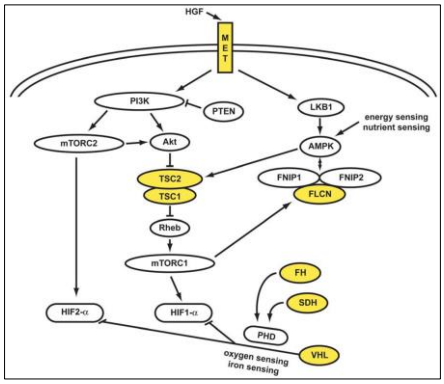
The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature. 2013;499:43-49

Clear Cell RCC

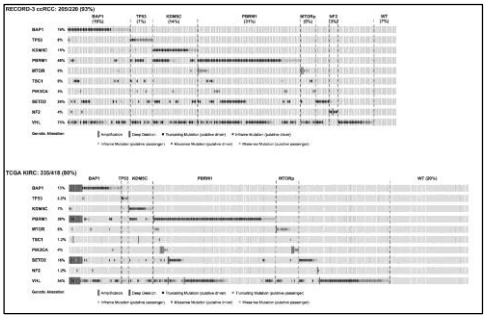
- VHL/ 3p LOH (90%)
- Deletion of 3p >90% (biallelic)- 3 genes
  - VHL: Tumor suppressor
  - PBRM1- chromatin remodeling complex
  - BAP1, SETD2, JARID1
- Epigenetic silencing in ~7%, mutually exclusive with mutation
- Inactivation of VHL serves as the fundamental driver event of human ccRCC

Casuscelli J et al. Molecular Classification of Renal Cell Carcinoma and Its Implication in Future Clinical Practice. Kidney Cancer 1 (2017) 3–13





Linehan WM et al. The genetic basis of kidney cancer: a metabolic disease. Nat Rev Urol. 2010;7(5):277-285

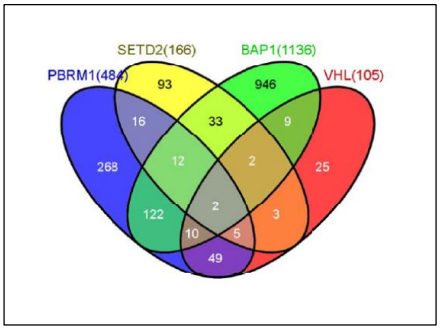


Casuscelli J et al. Molecular Classification of Renal Cell Carcinoma and Its Implication in Future Clinical Practice. Kidney Cancer 1 (2017) 3–13

Common Genetic Alterations in ccRCCs

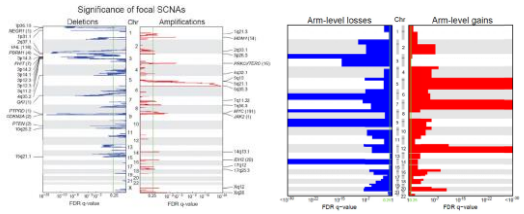
Mutations in 93% of ccRCC	Percentage of cases	Clinical Impact	
VHL	>70%	Diagnostic	No prognostic impact
PBRM1	~ 40%		Longer survival on MTORI
BAP1	~ 15-20%		High grade, poor outcomes on VEGFR TKI/ MTOR Inhibitor
SETD2	~ 7-11%		Worse survival, associated with metastases
KDM5C	~ 14%		Longer survival on VEGF TKI
TP53	2.2 – 8%		High grade, decreased survival
PIK3CA			Targetable
MTOR	~ 5%		Response to MTORI, mutations in metastases better response than mutations in primary
TSC1			Targetable
NF2	~ 3%		Targetable

Casuscelli J et al. Molecular Classification of Renal Cell Carcinoma and Its Implication in Future Clinical Practice. Kidney Cancer 1 (2017) 3–13
Hseieh JJ et al. Genomic classifications of renal cell carcinoma: a critical step towards the future application of personalized kidney cancer care with pan-omics precision. J Pathol 2018; 244: 525–537



The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature. 2013;499:43-49

Copy Number Changes: ccRCCs



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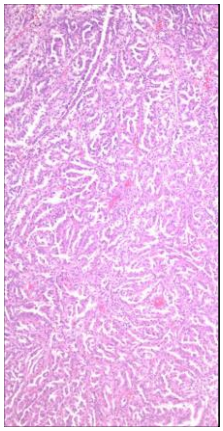
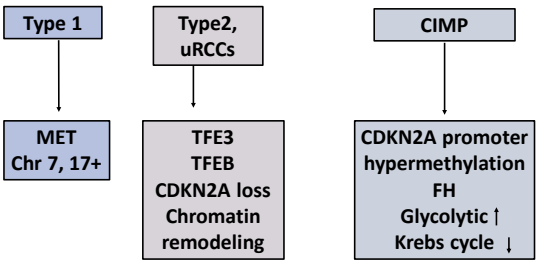


Papillary RCC

- 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

TCGA: pRCC



The Cancer Genome Atlas Research Network. Comprehensive Molecular Characterization of Papillary Renal-Cell Carcinoma. N Engl J Med 2016;374:135-45.

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

Hseieh JJ et al. Genomic classifications of renal cell carcinoma: a critical step towards the future application of personalized kidney cancer care with pan-omics precision. J Pathol 2018; 244: 525–537

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

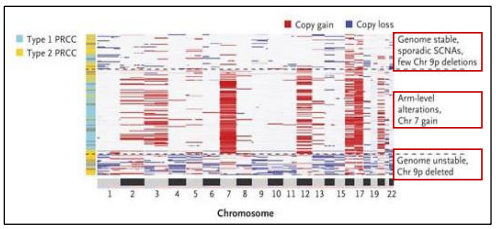
The Cancer Genome Atlas Research Network. Comprehensive Molecular Characterization of Papillary Renal-Cell Carcinoma. N Engl J Med 2016;374:135-45.

Molecular Differences Between Type 1 &2 pRCCs

		Type 1	Type 2
NF2	Hippo signaling pathway	2.8%	10.0%
SMARCB1, PBRM1	SWI/SNF complex	19.7%	26.7%
SETD2, KDM6A, BAP1	Chromatin remodeling pathways	35.2%	38.3%

The Cancer Genome Atlas Research Network. Comprehensive Molecular Characterization of Papillary Renal-Cell Carcinoma. N Engl J Med 2016;374:135-45.

Copy Number Changes: pRCCs



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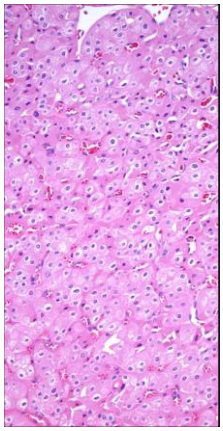


Chromophobe RCC

- 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

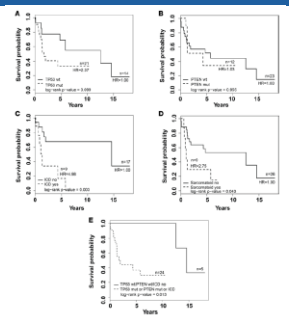
Davis CF et al. The somatic genomic landscape of chromophobe renal cell carcinoma.Cancer Cell. 2014; 26(3): 319–330

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

Casuscelli J et al. Genomic landscape and evolution of metastatic chromophobe renal cell carcinoma.JCI Insight. 2017;2(12):e92688

Aggressive Chromophobe RCCs



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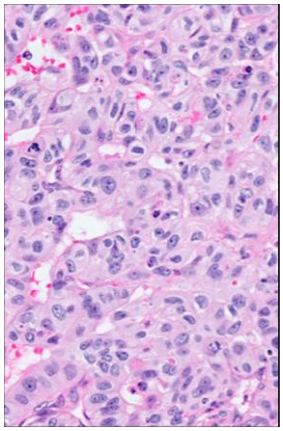

Unclassified RCC

- 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%)
  - Better outcomes, therapeutic target
  - 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%)

Chen Y-B et al. Molecular analysis of aggressive renal cell carcinoma with unclassified histology reveals distinct subsets. Nat Commun. 2016;7:13131

Other RCC Subtypes

RCC Subtype	Molecular Alterations
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

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Genomic correlates with clinical outcomes

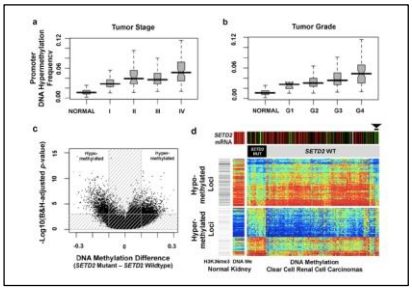
DNA methylation patterns

- 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

KIRP	Morphological pattern	Outcomes
Cluster 1	Type 1, MET mutation, Chr 7+	Low tumor stage. Best survival
Cluster 2a	Type 2	Low tumor stage, Best survival
Cluster 2b	Type2, unclassified papillary RCC,	High tumor stage. Poor survival
Cluster 2c	CIMP tumor subtype NRF2-ARE pathway alterations	Worst survival

Chen F et al. Multilevel Genomics-Based Taxonomy of Renal Cell Carcinoma. Cell Reports 2016;14, 2476–2489

DNA Methylation



The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature. 2013;499:43-49

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

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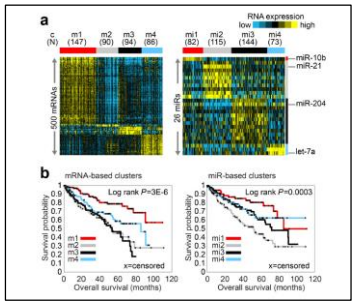
miRNA: ccRCC



The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature. 2013;499:43-49

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

The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature. 2013;499:43-49

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

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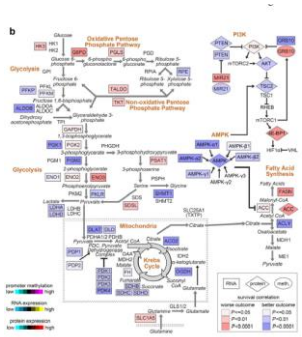

- Worse survival:
  - Pentose phosphate pathway
  - Fatty acid synthesis
  - PI3K pathway genes
- Better survival
  - AMPK
  - Krebs cycle
  - PI3K pathway inhibitor genes

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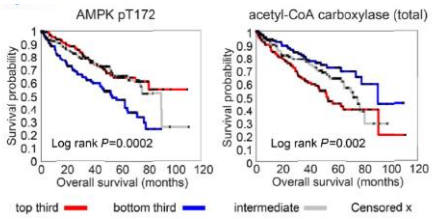

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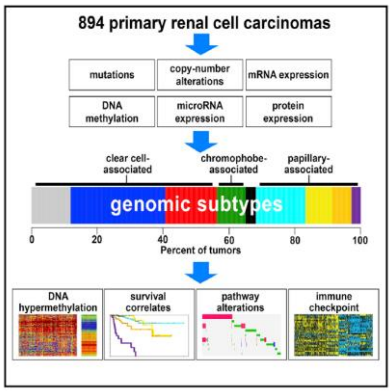
Integrated Multi-omics across RCC subtypes



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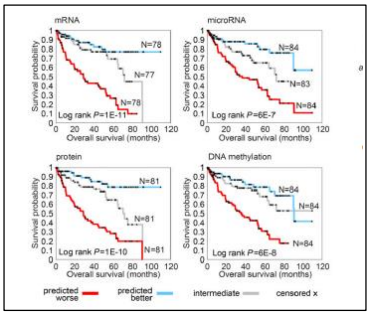

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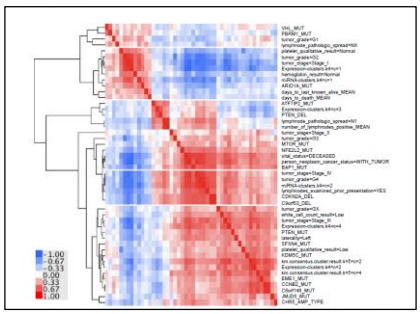

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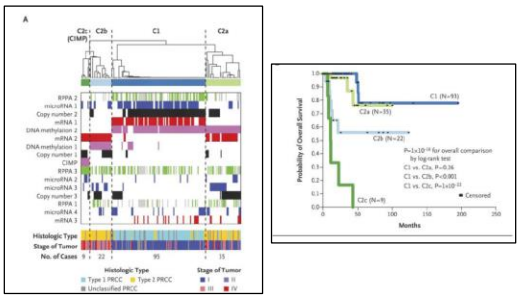

The Cancer Genome Atlas Research Network. Comprehensive Molecular Characterization of Papillary Renal-Cell Carcinoma. N Engl J Med 2016;374:135-45.

9 Molecular Subtypes

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

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Casuscelli J et al. Molecular Classification of Renal Cell Carcinoma and Its Implication in Future Clinical Practice. Kidney Cancer 1 (2017) 3–13

Immunotherapy Biomarkers

PD-L1/PD-1 Inhibitors

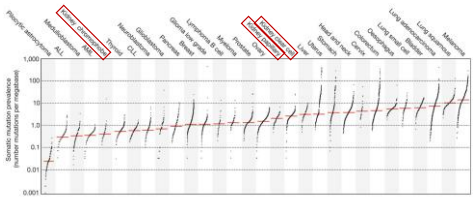
- ccRCC: High expression of several immunotherapy gene targets
  - Greater levels of immune infiltrates
- Many poor risk and Sarcomatoid tumors
  - High levels of PD-L1 expression
  - Greatest relative benefit with nivolumab over everolimus
- CheckMate 025 trial: Higher PD-L1 expression
  - Poor survival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

Chen F et al. Multilevel Genomics-Based Taxonomy of Renal Cell Carcinoma. Cell Reports 2016;14, 2476–2489
Özdemir BC et al. Current and Future Applications of Novel Immunotherapies in Urological Oncology: A Critical Review of the Literature. Eur Urol Focus. 2018 Apr;4(3):442-454

PD-1/PD-L1 Challenges

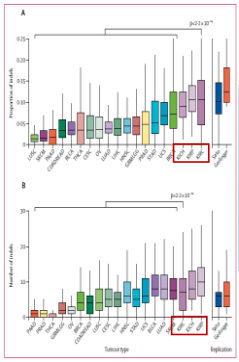
- Different antibodies
- Immune infiltrating cells evaluated
- Intratumoral and intertumoral heterogeneity of PD-L1 expression
- Temporal evolution of PD-L1 status during the development of treatment resistance
- Variation in PD-L1 expression according to the level of tissue hypoxia

Mutational Load



RCC: Low mutational burden

Alexandrov LB et al. Signatures of mutational processes in human cancer. Nature 2013; 500:415



- 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

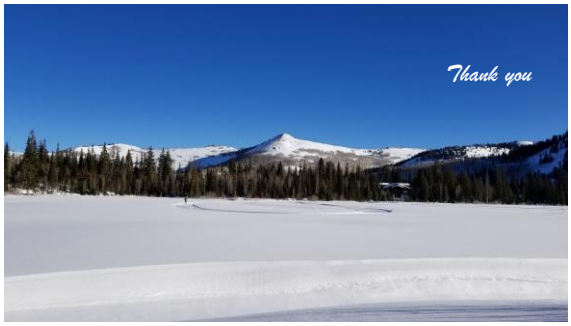
Turajlic S et al. Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: a pan-cancer analysis. Lancet Oncol. 2017 Aug;18(8):1009-1021

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