Molecular Subtypes of Renal Cell Carcinoma

Deepika Sirohi, MD

University of Utah and ARUP Laboratories

2019 Annual Park City Anatomical Pathology Update

- 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

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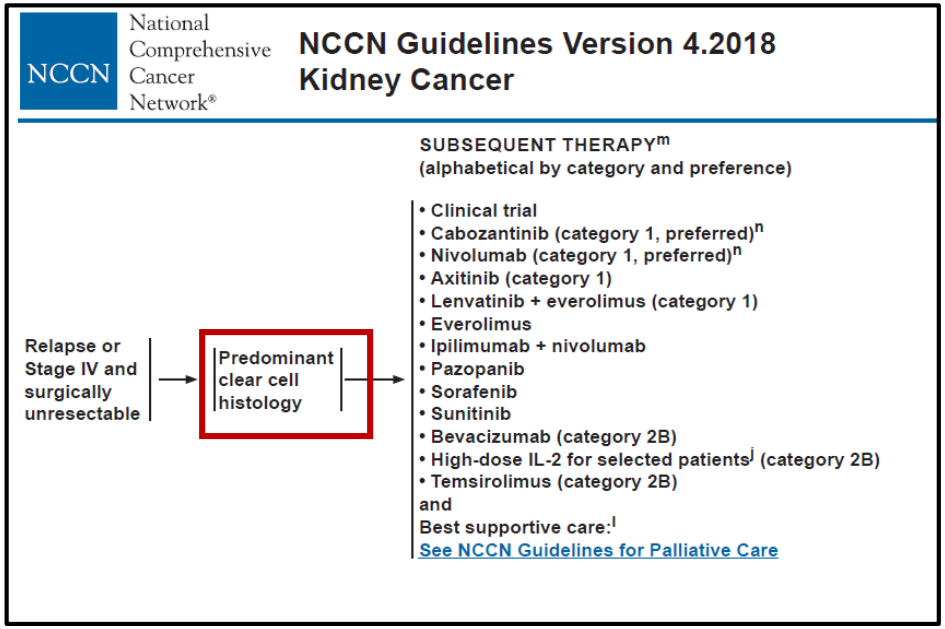
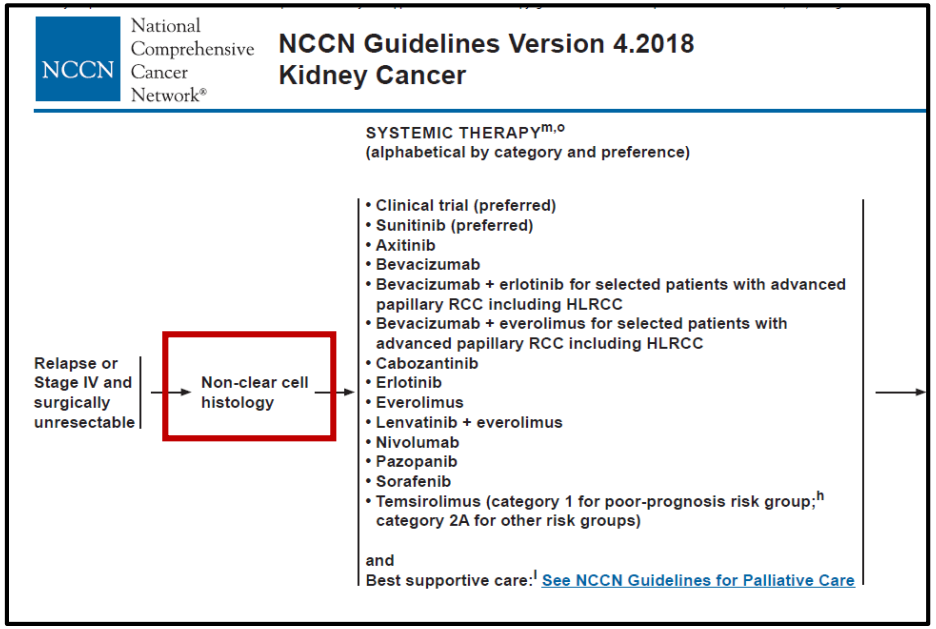
RCC: Treatment Strategies

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

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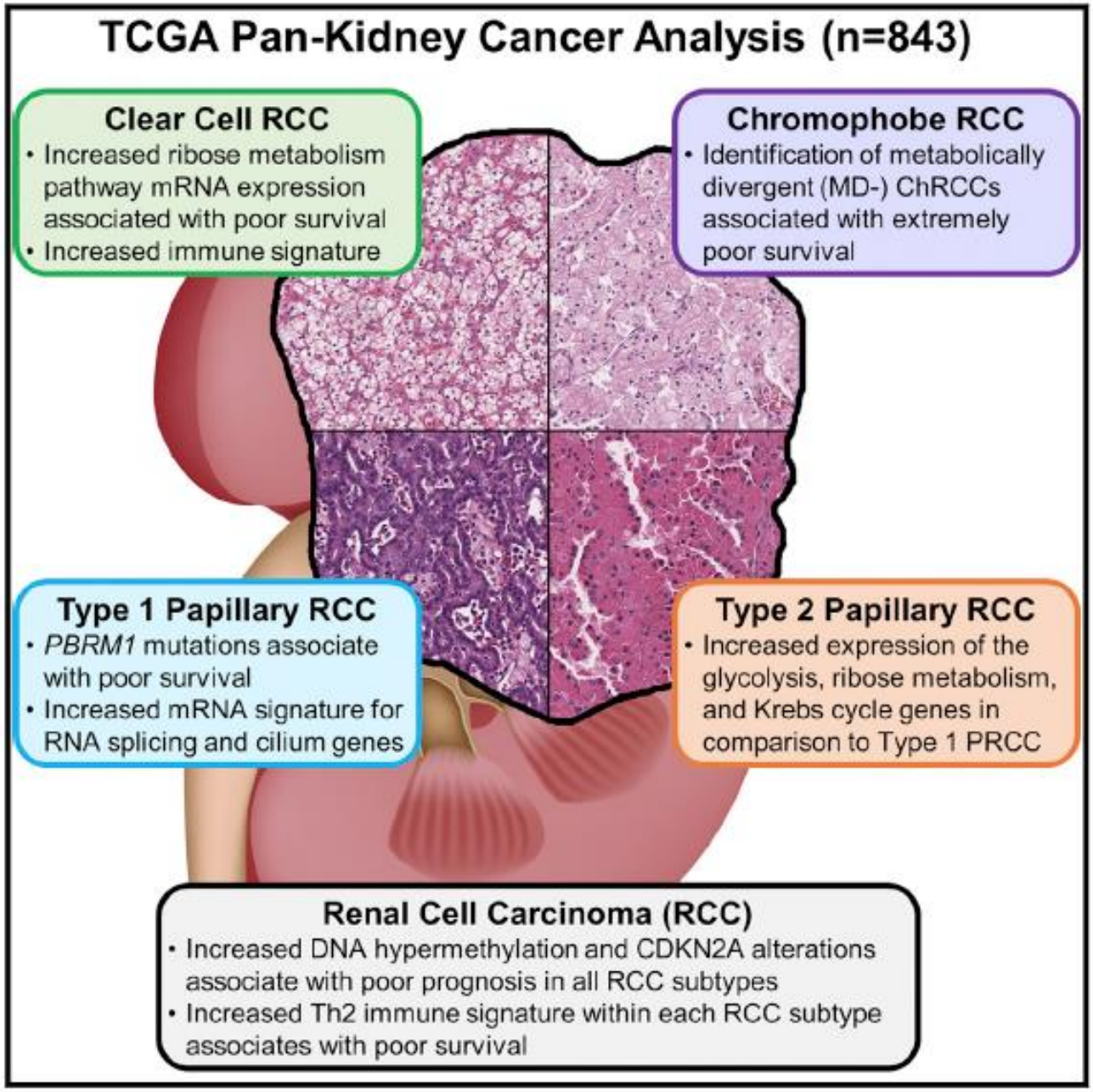
Genomic Landscape of RCC

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



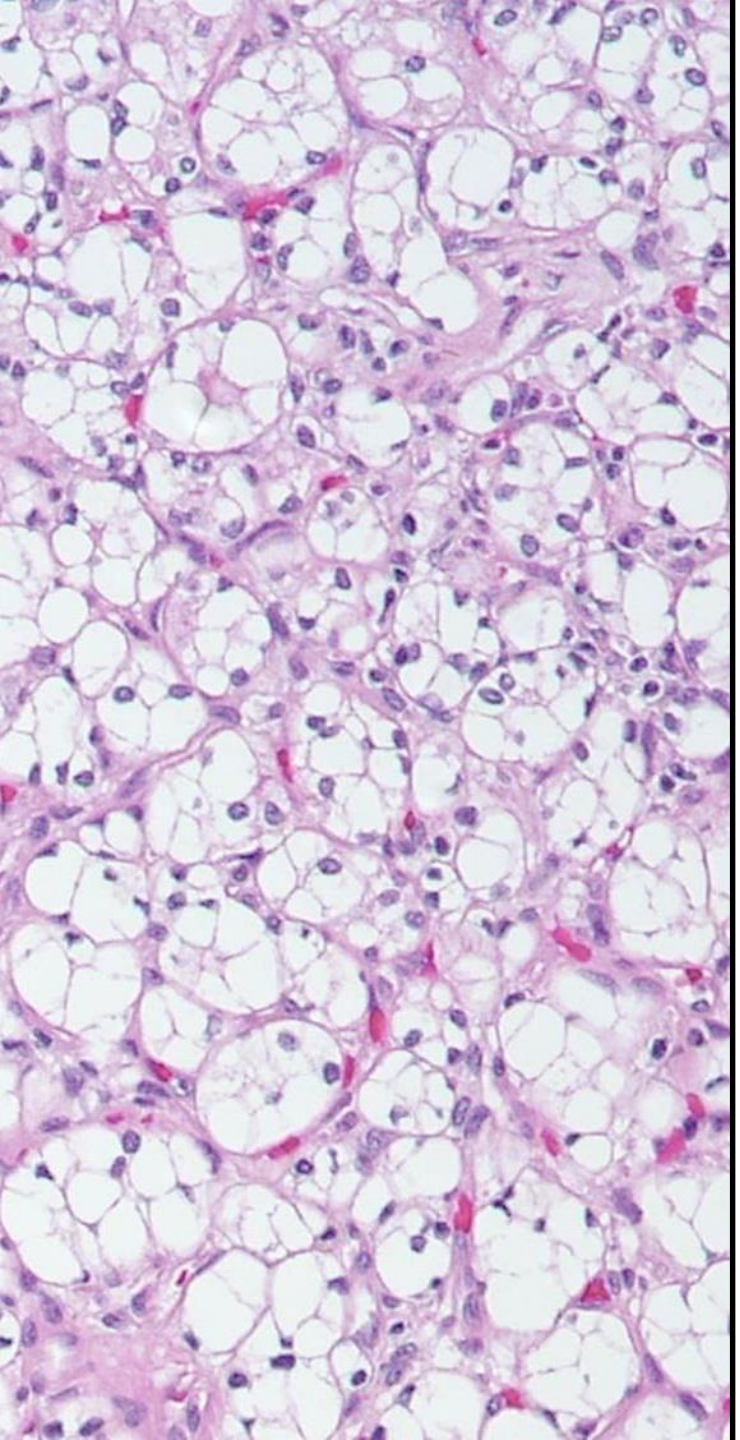
Ricketts et al. The Cancer Genome Atlas Comprehensive Molecular Characterization of Renal Cell Carcinoma. Cell Reports 2018;23:313–326

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

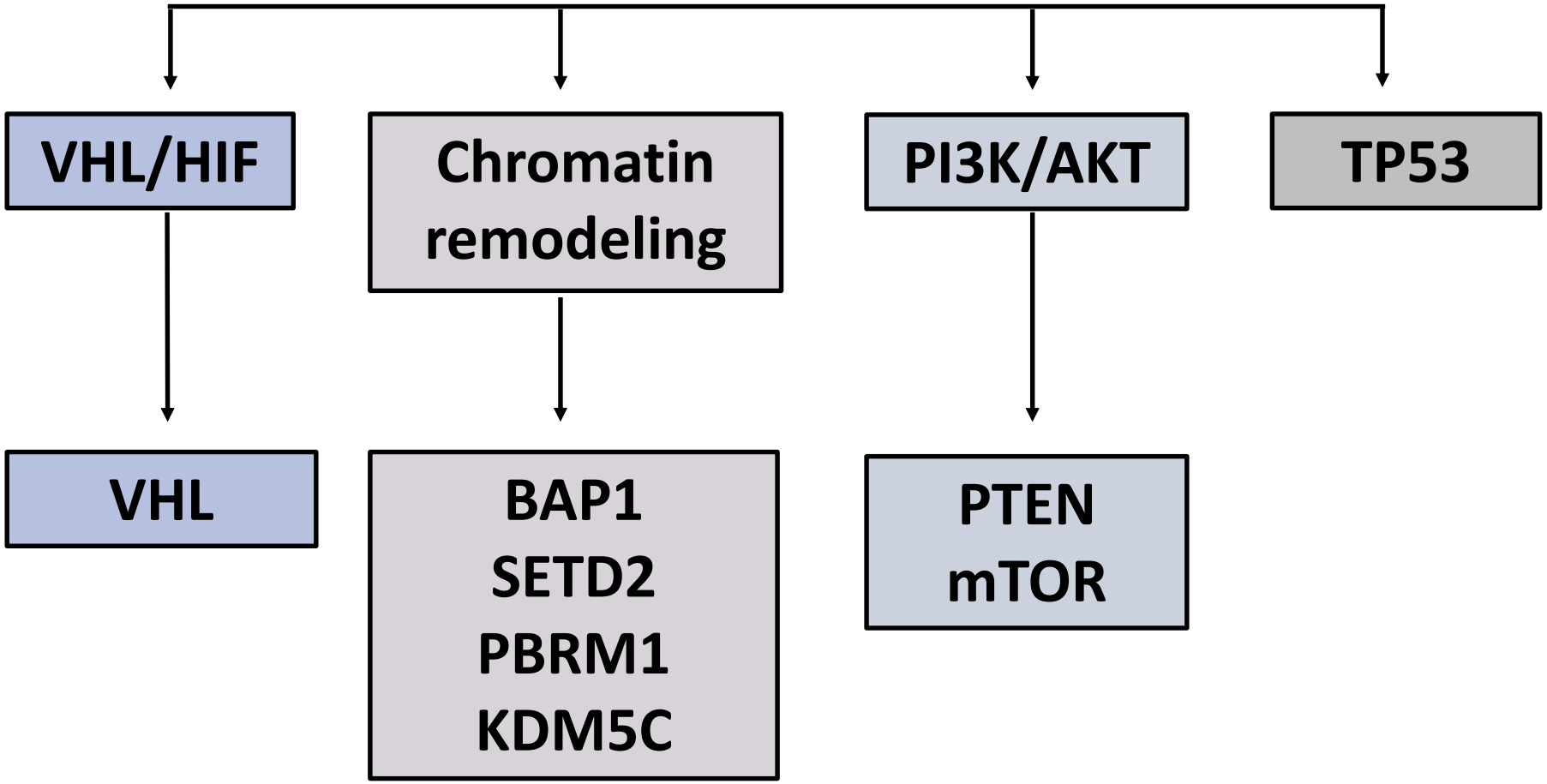
Histopathology and Molecular Pathology



Clear Cell RCC

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

TCGA: ccRCC

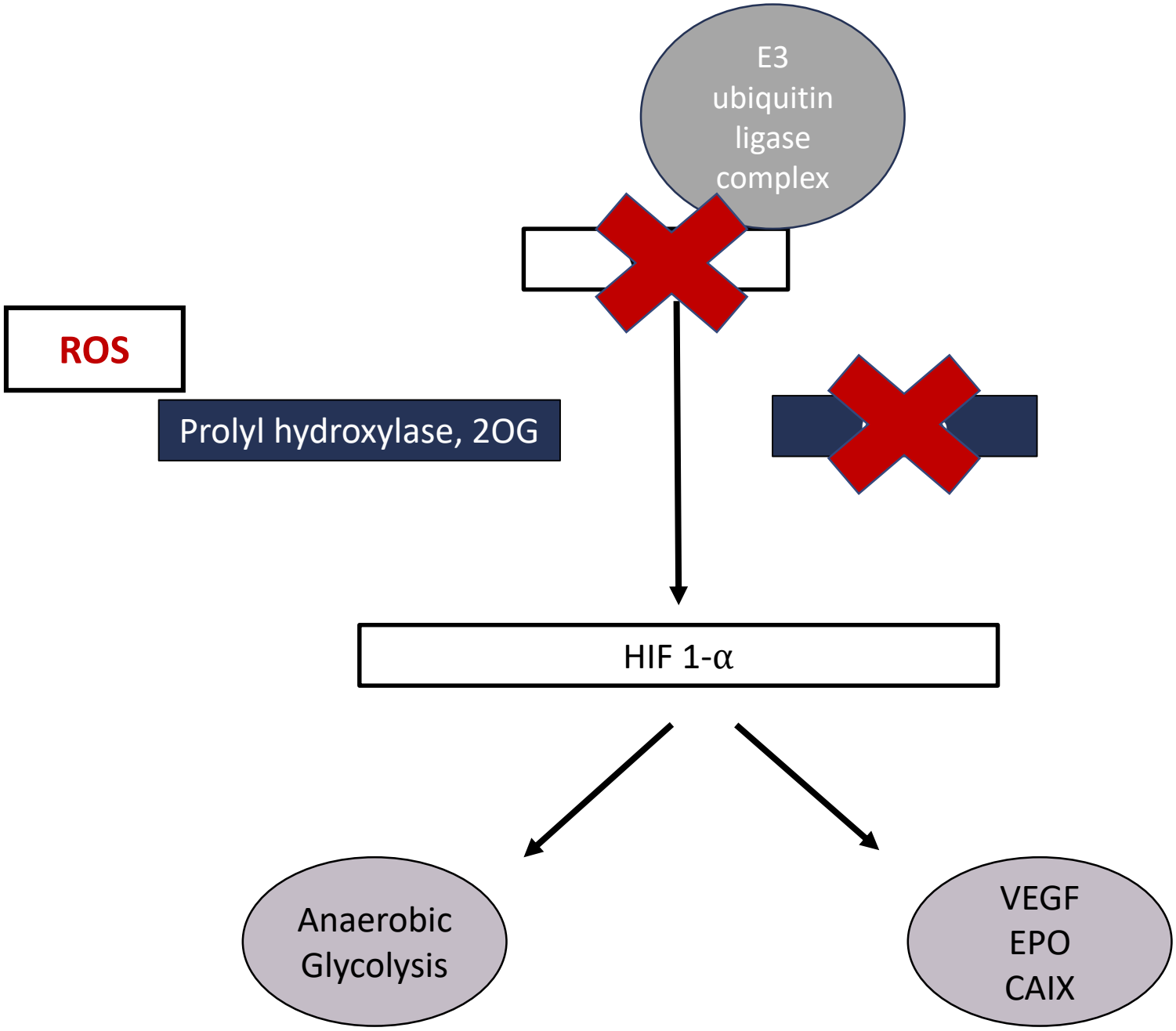


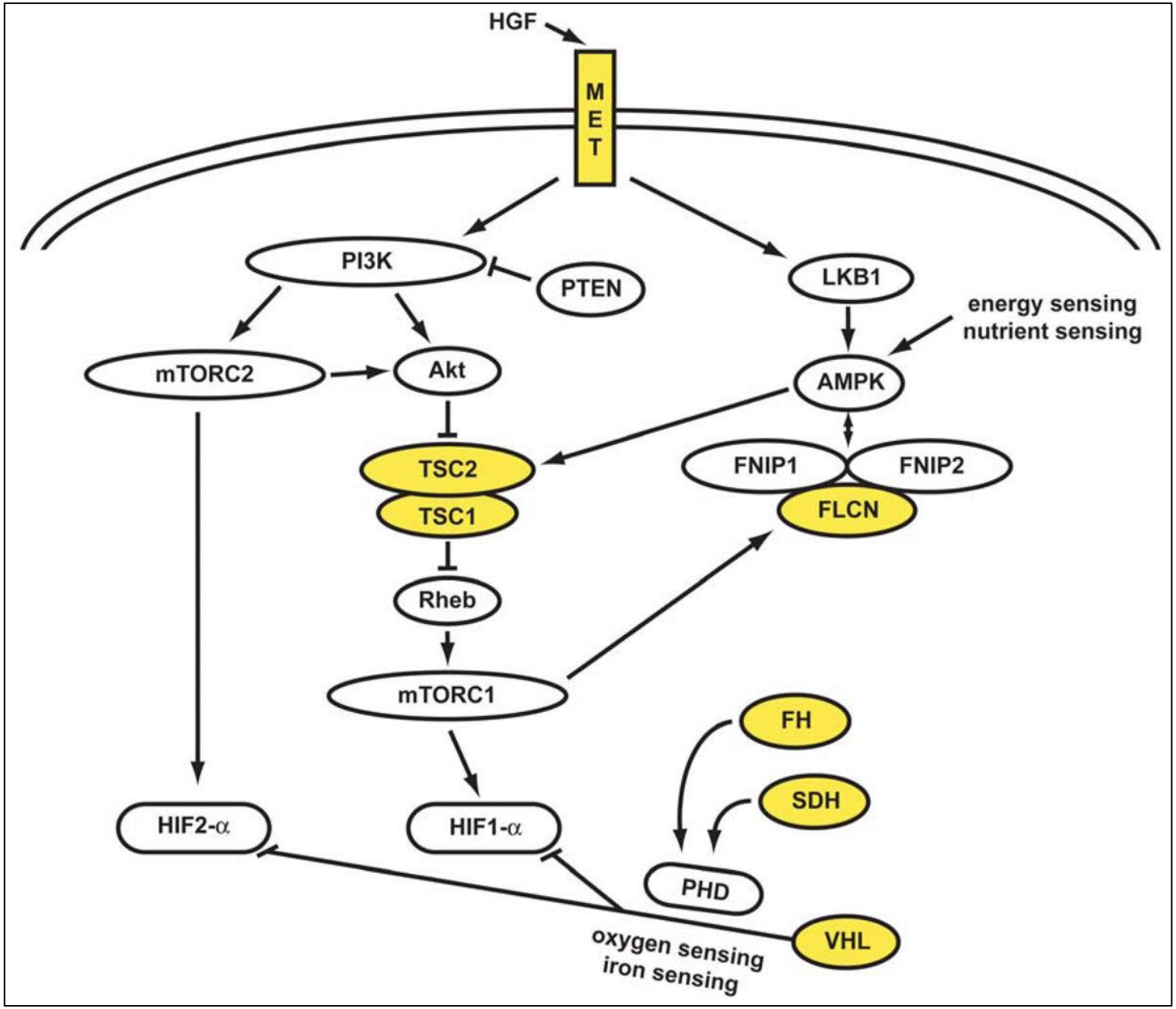
The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature. 2013;499:43-49

Clear Cell RCC

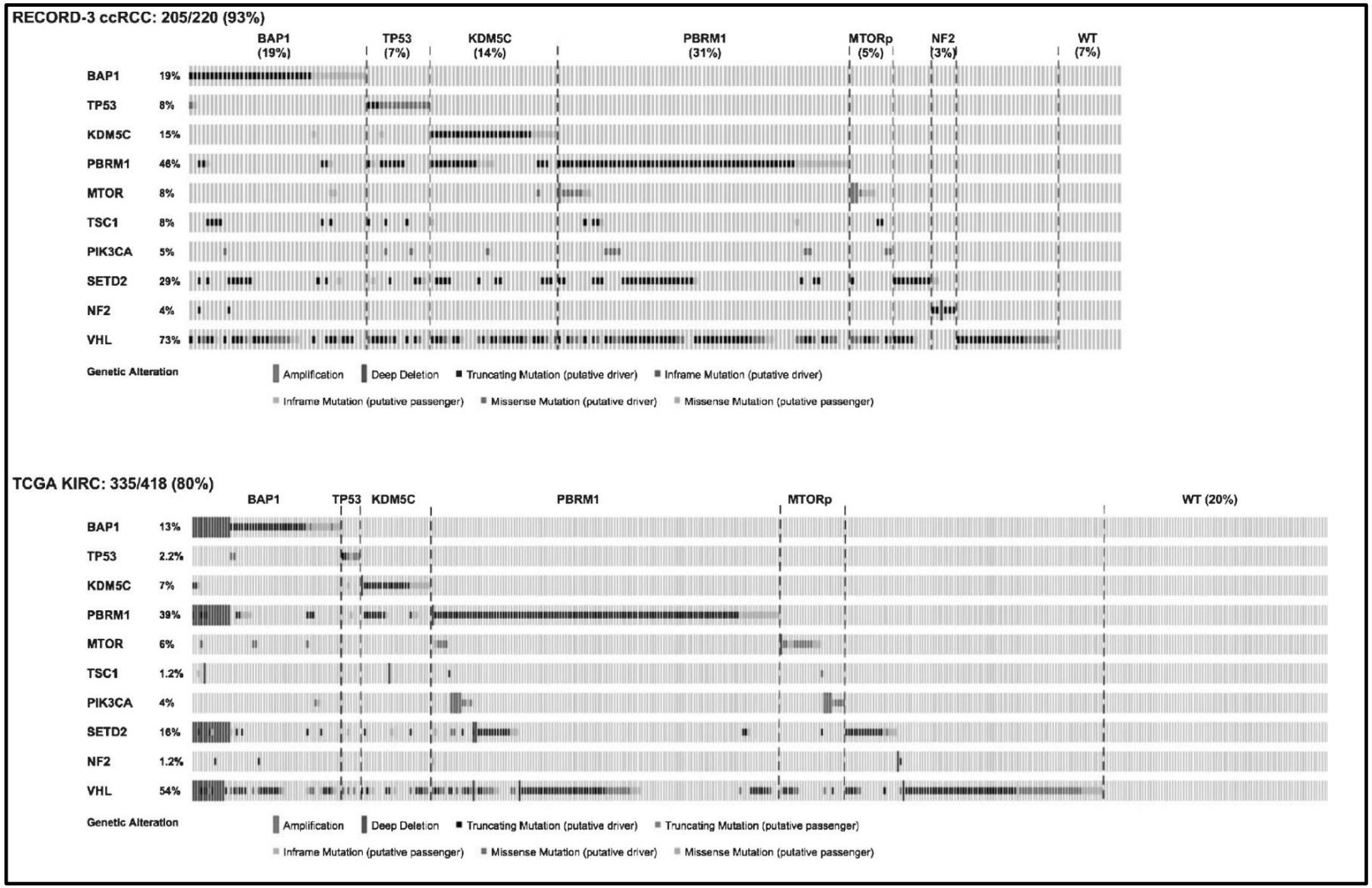
- VHL/ 3p LOH (90%)
- Deletion of 3p >90% (biallelic)- 3 genes
  - VHL: Tumor suppressor
  - PBRM1- chromatin remodeling complex
  - BAP1, SETD2, JARID1
- Epigenetic silencing in ~7%, mutually exclusive with mutation
- Inactivation of VHL serves as the fundamental driver event of human ccRCC

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


E3
ubiquitin
ligase
complex
ROS
Prolyl hydroxylase, 2OG
HIF 1-α
Anaerobic
Glycolysis
VEGF
EPO
CAIX



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



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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

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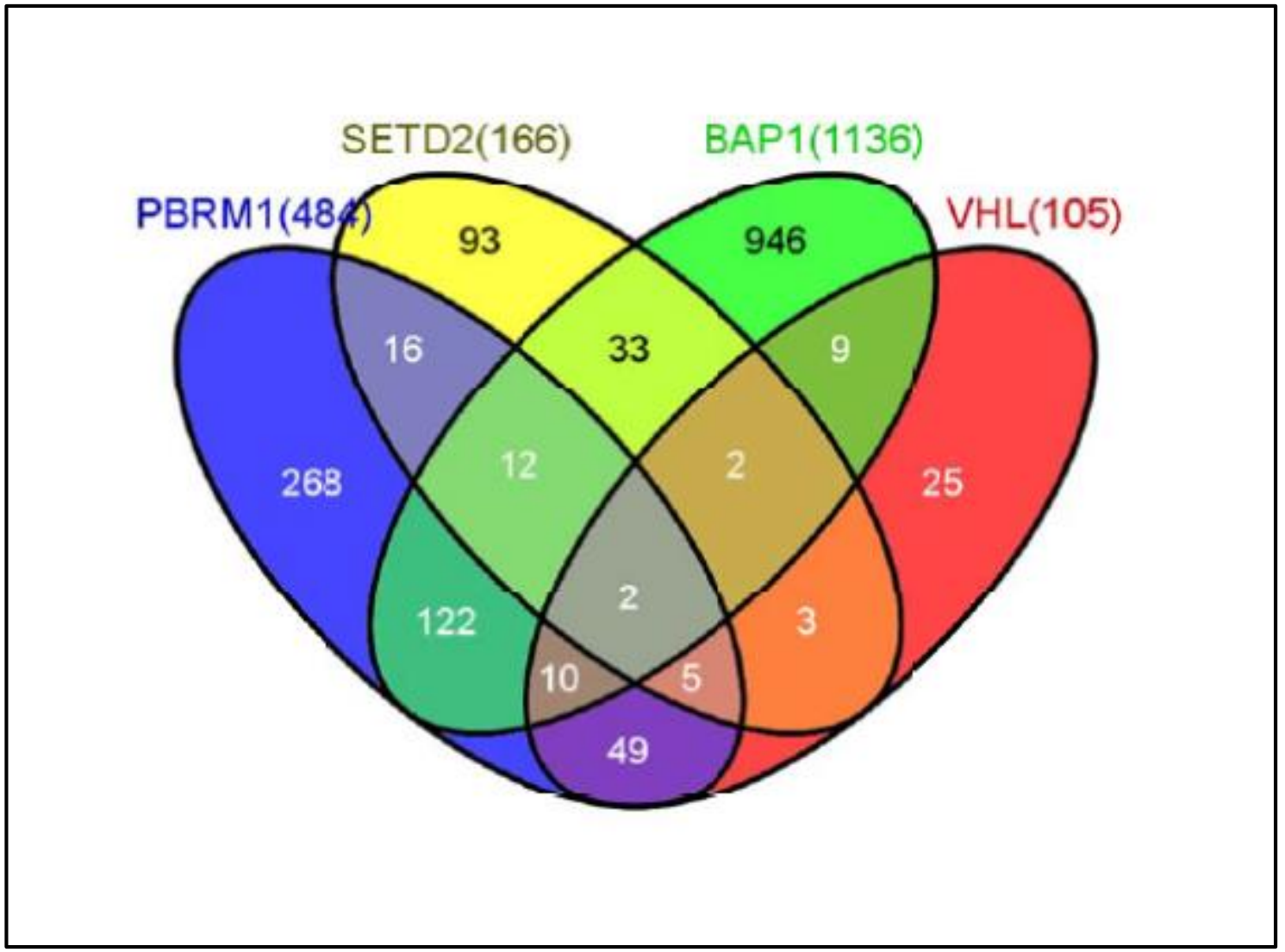
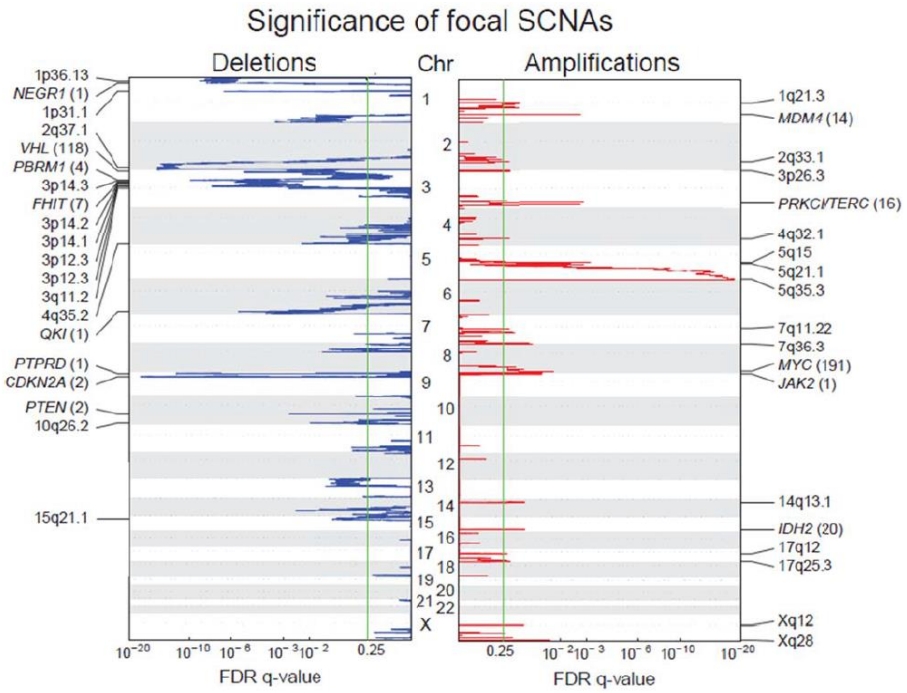
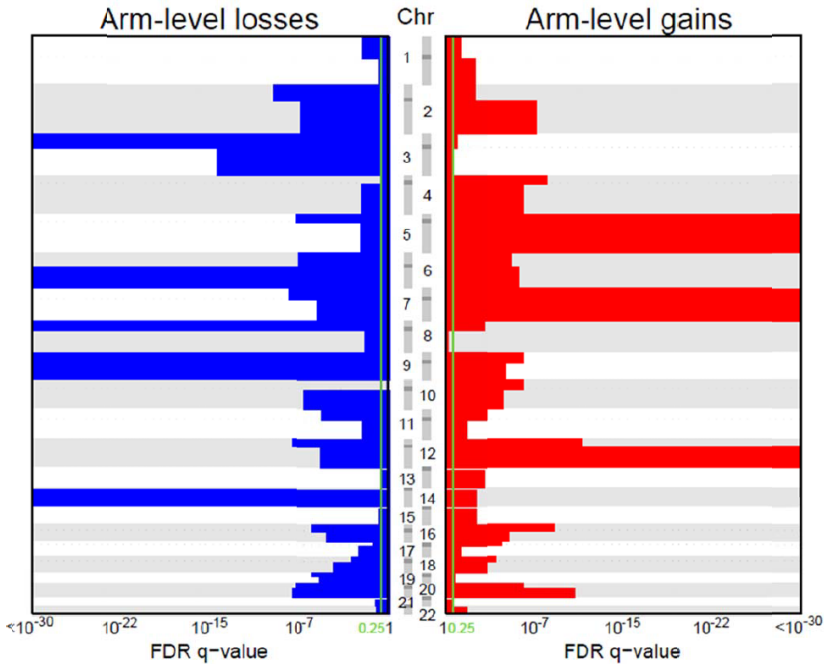
Hseieh JJ et al. Genomic classifications of renal cell carcinoma: a critical step towards the future application of personalized kidney cancer care with pan-omics precision. J Pathol 2018; 244: 525–537



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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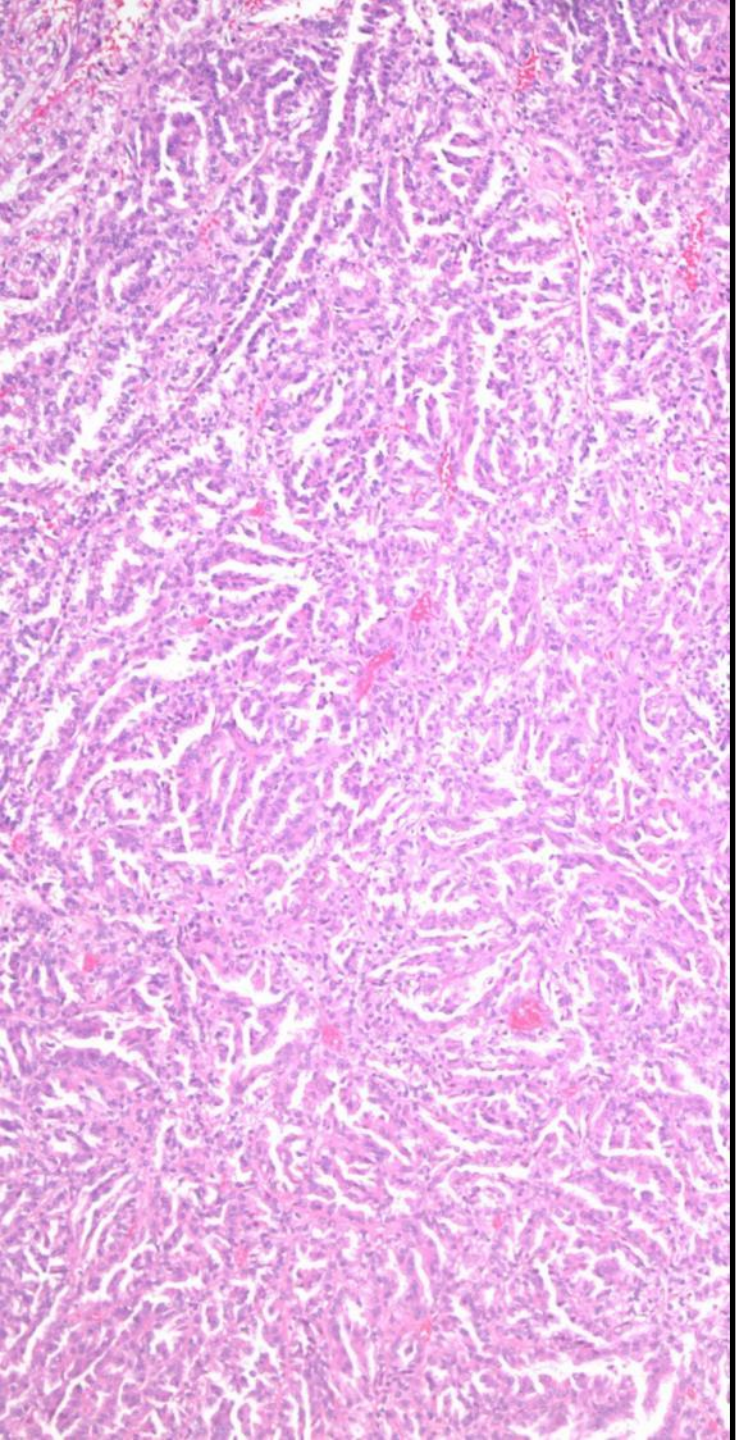
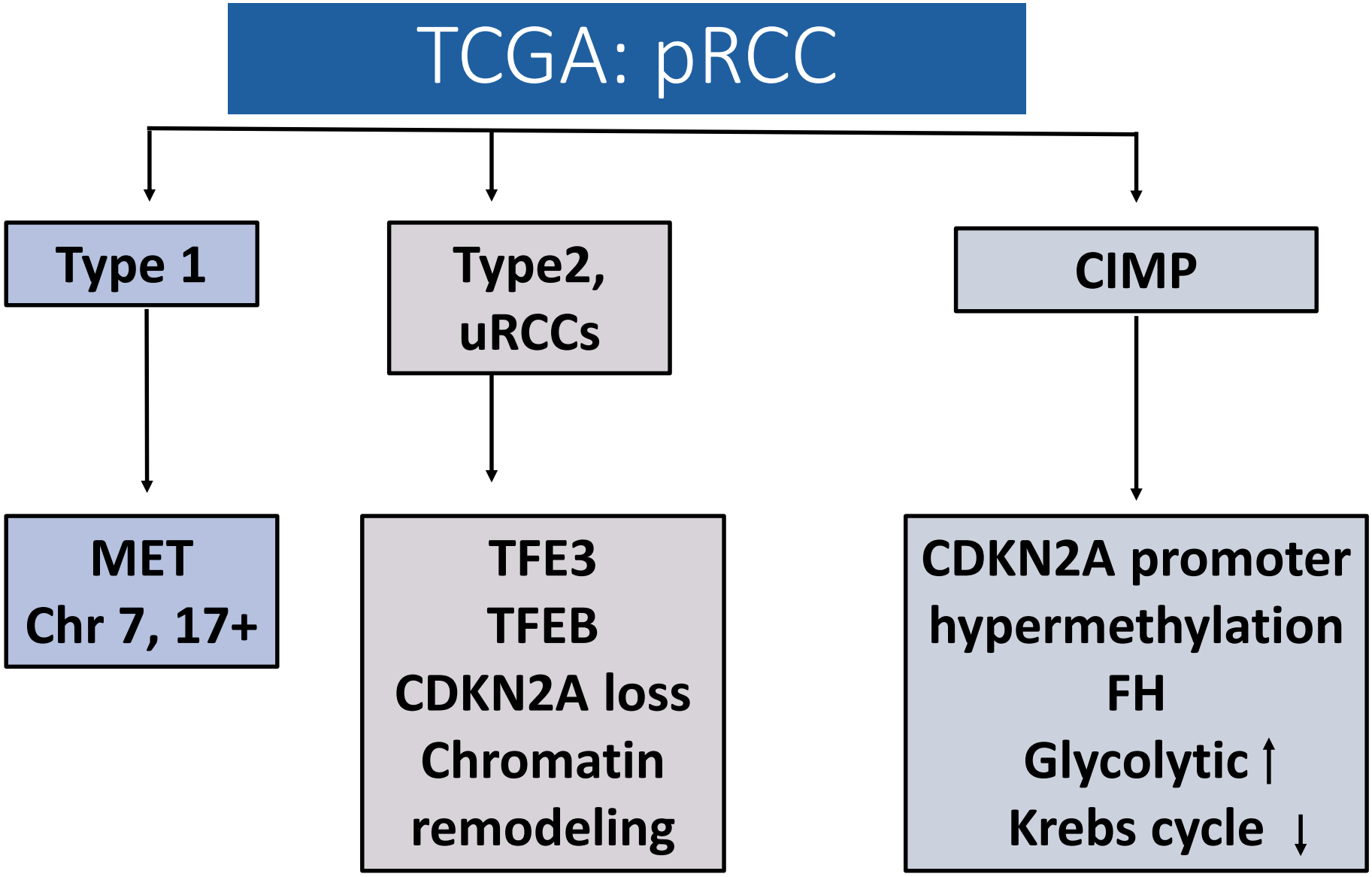


Papillary RCC

- 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



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.

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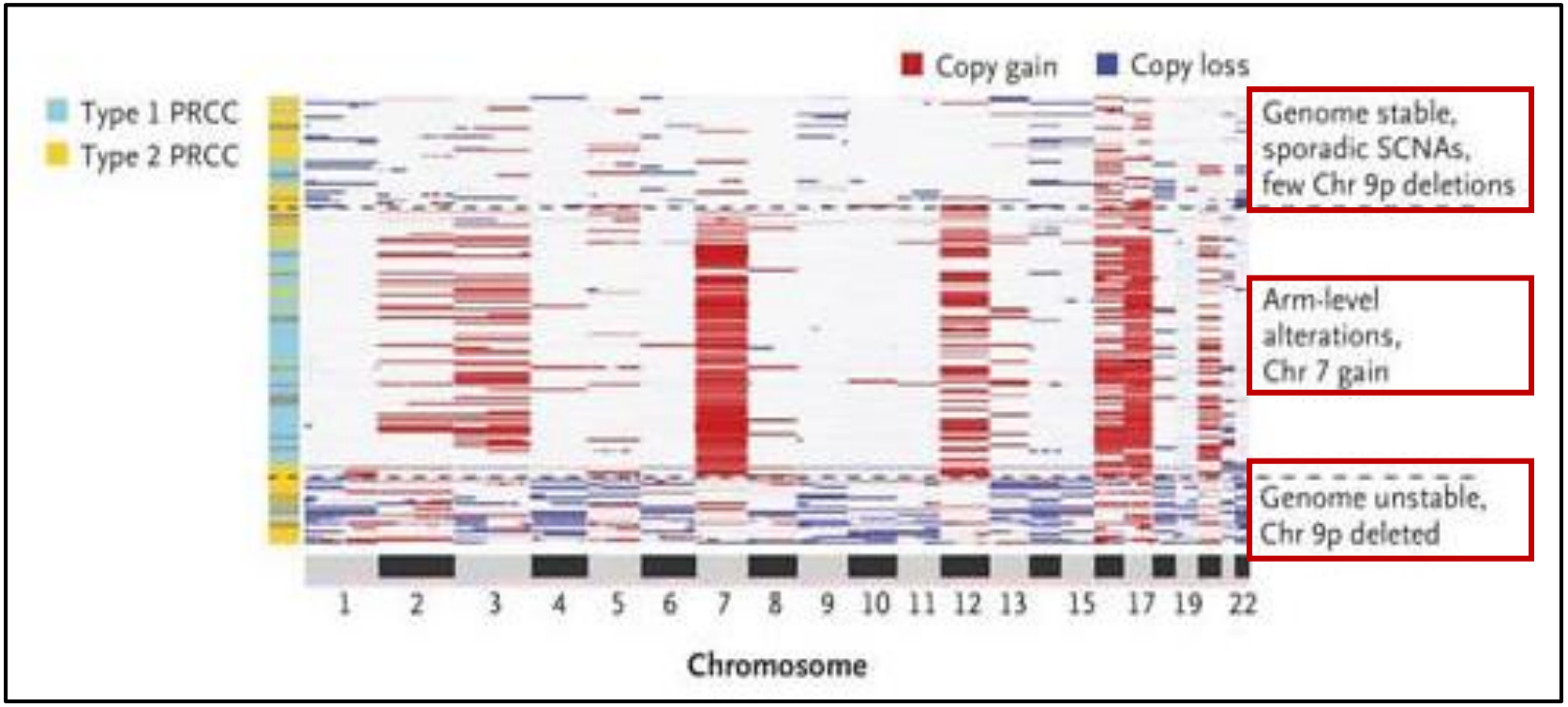

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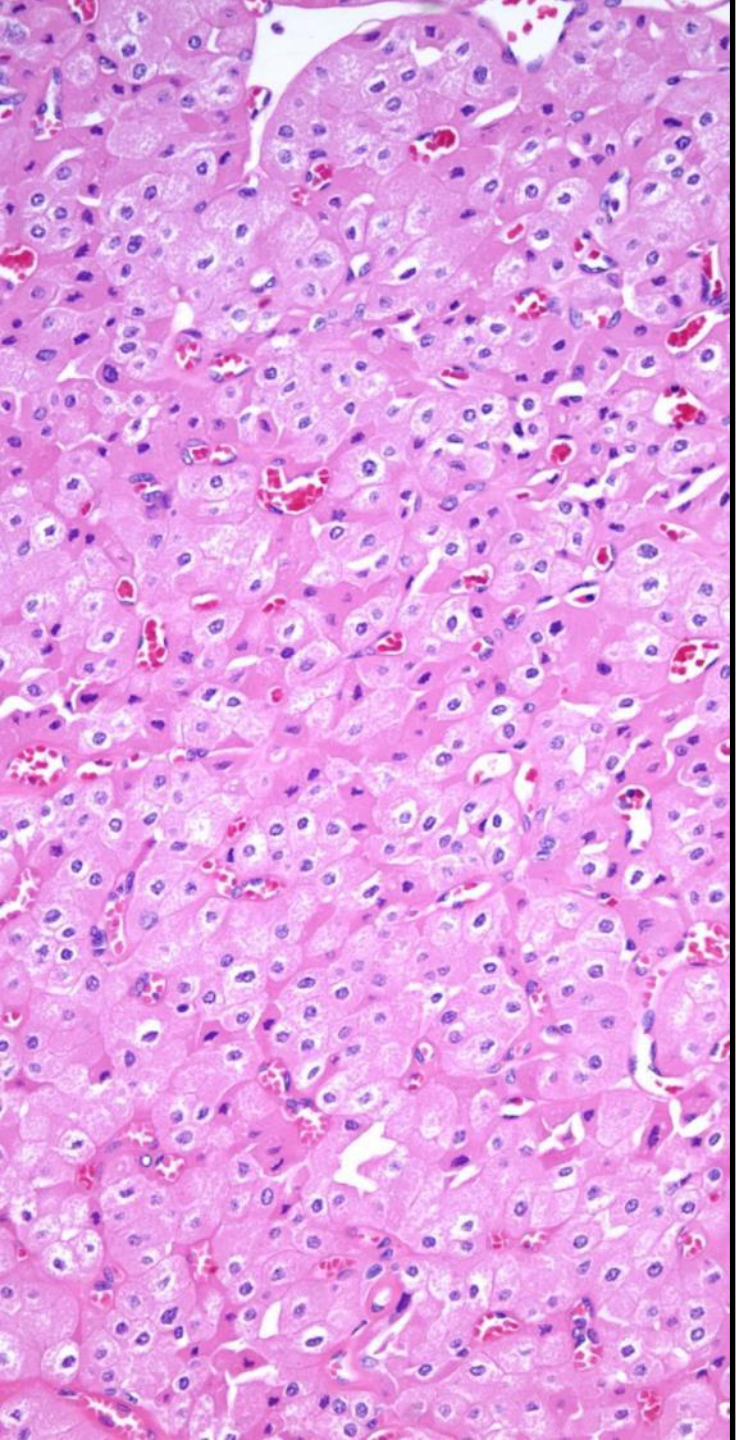


Chromophobe RCC

- 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

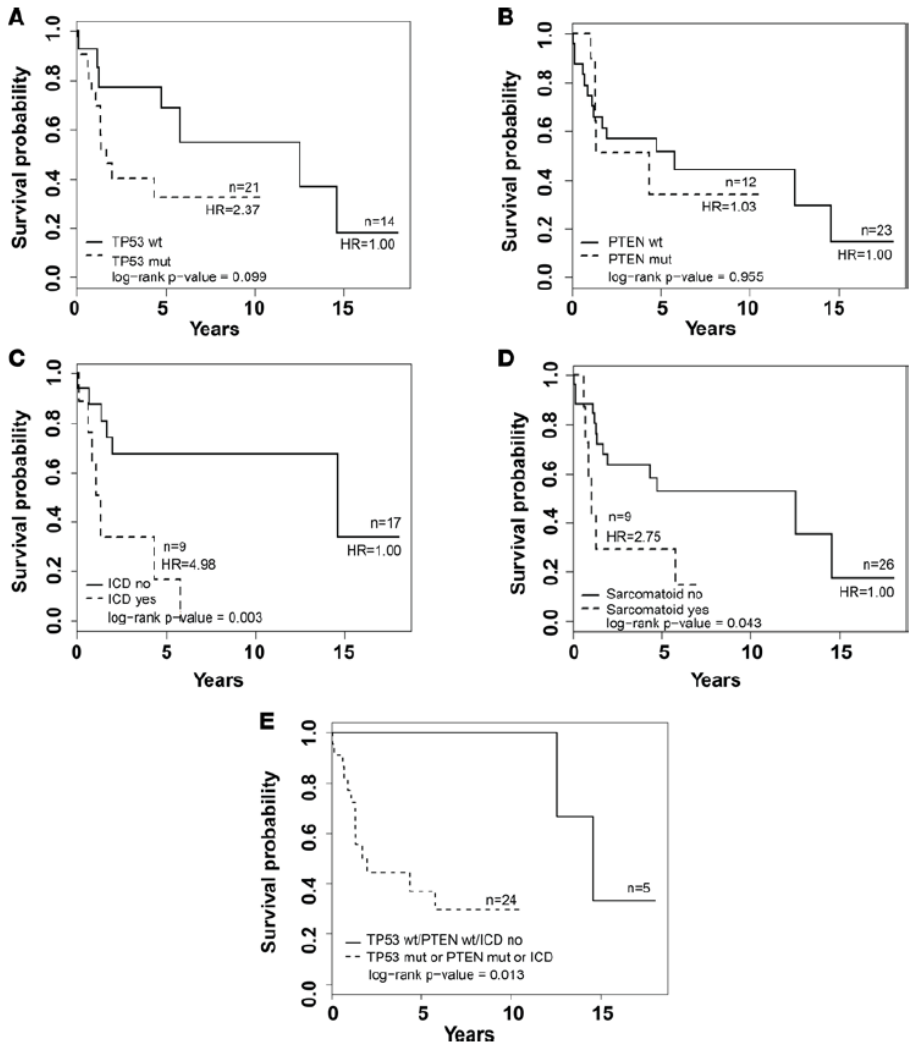
Davis CF et al. The somatic genomic landscape of chromophobe renal cell carcinoma.Cancer Cell. 2014; 26(3): 319–330

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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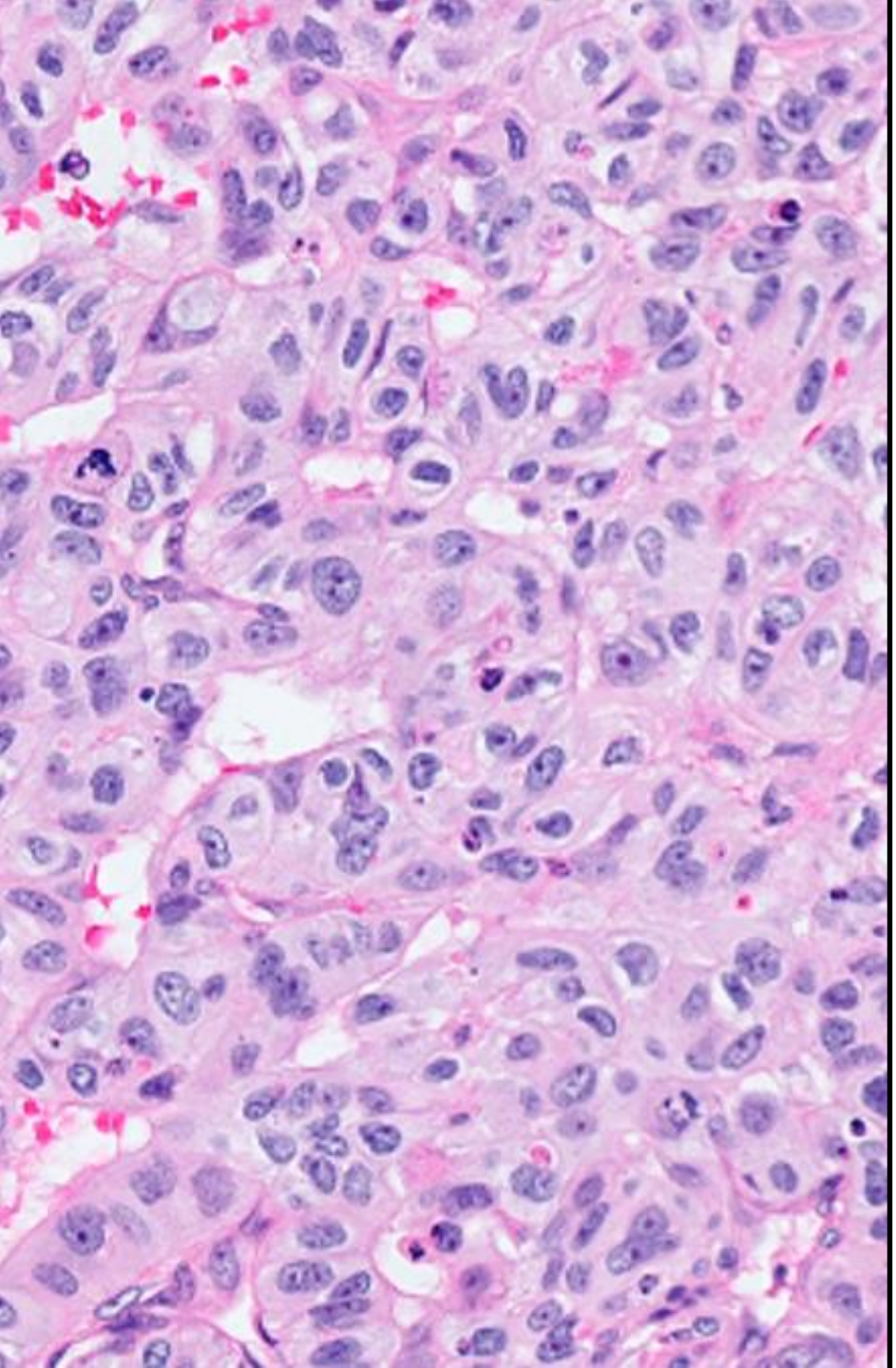

Unclassified RCC

- 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

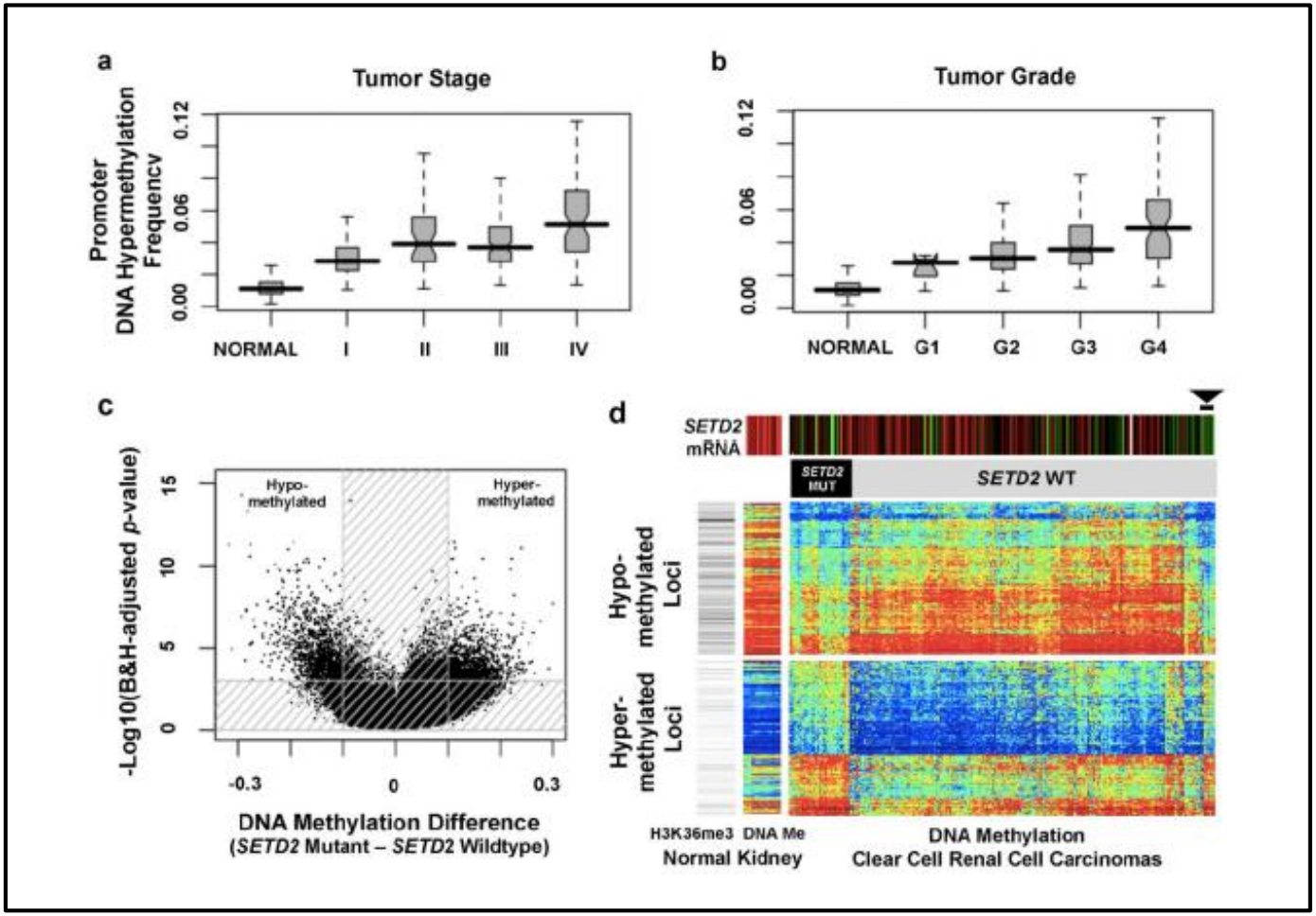
DNA methylation patterns

- 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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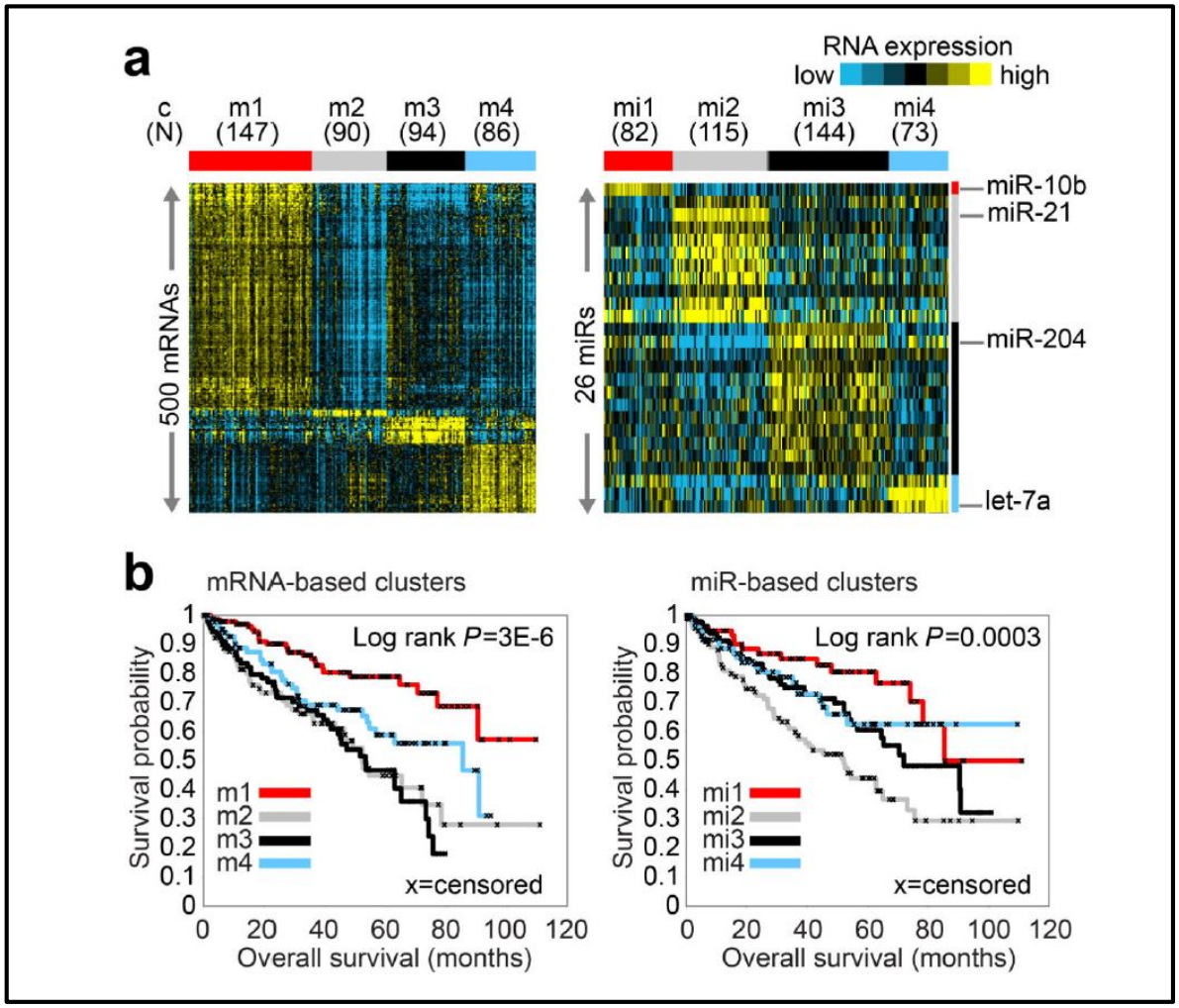
miRNA: ccRCC



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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

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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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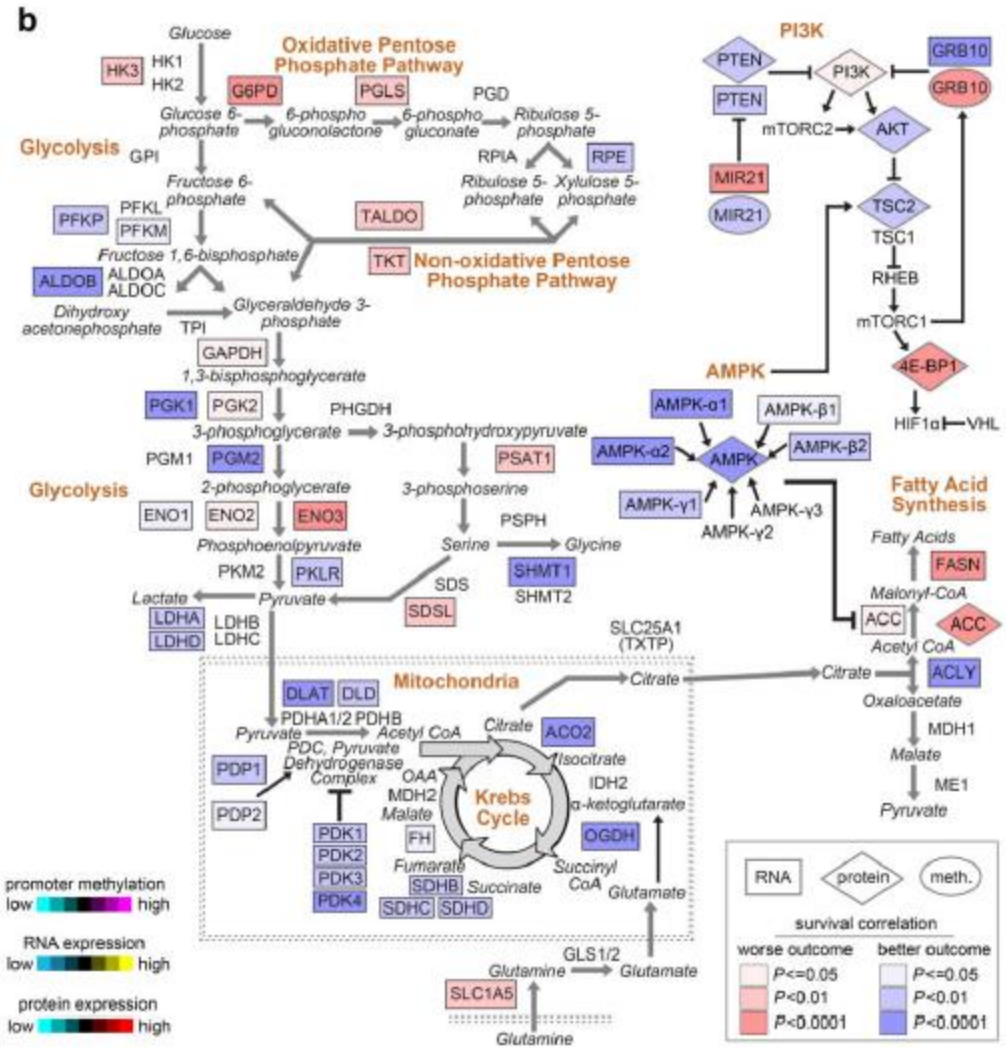

- Worse survival:
  - Pentose phosphate pathway
  - Fatty acid synthesis
  - PI3K pathway genes
- Better survival
  - AMPK
  - Krebs cycle
  - PI3K pathway inhibitor genes

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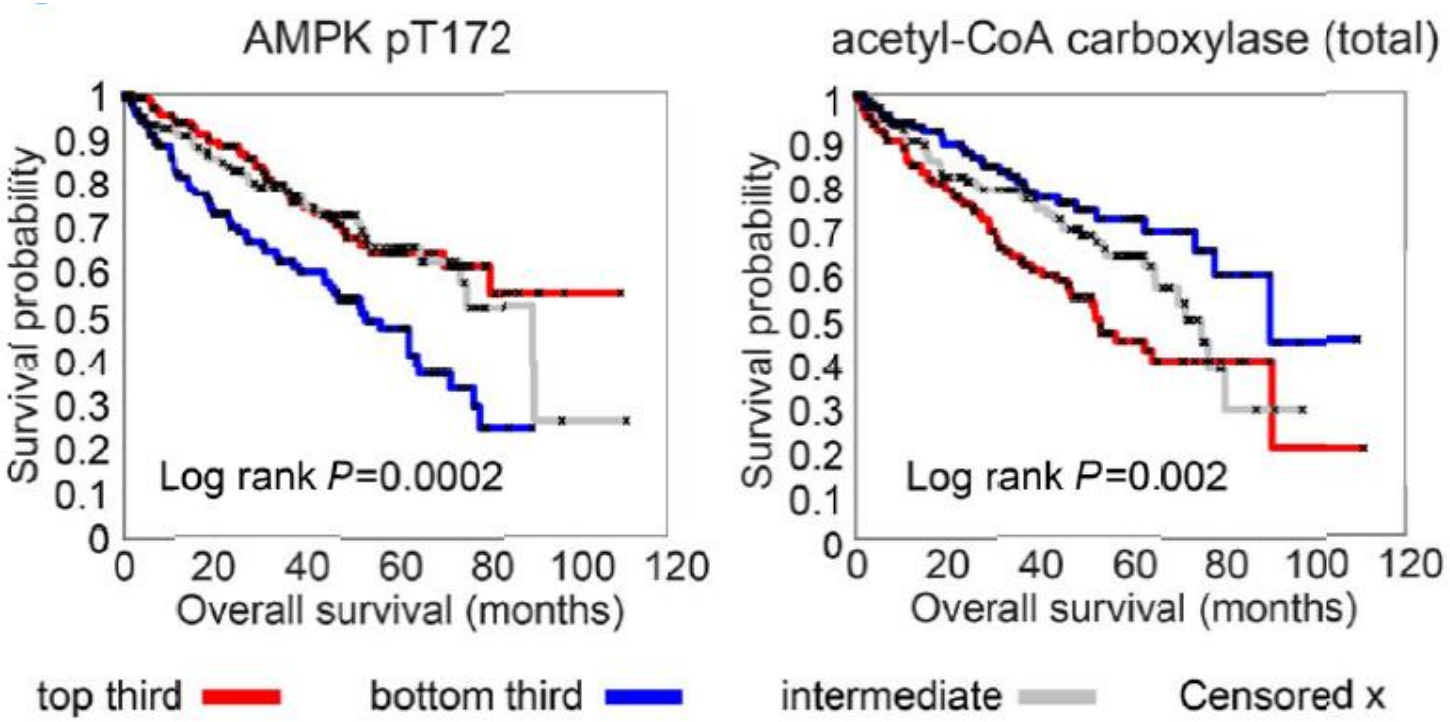

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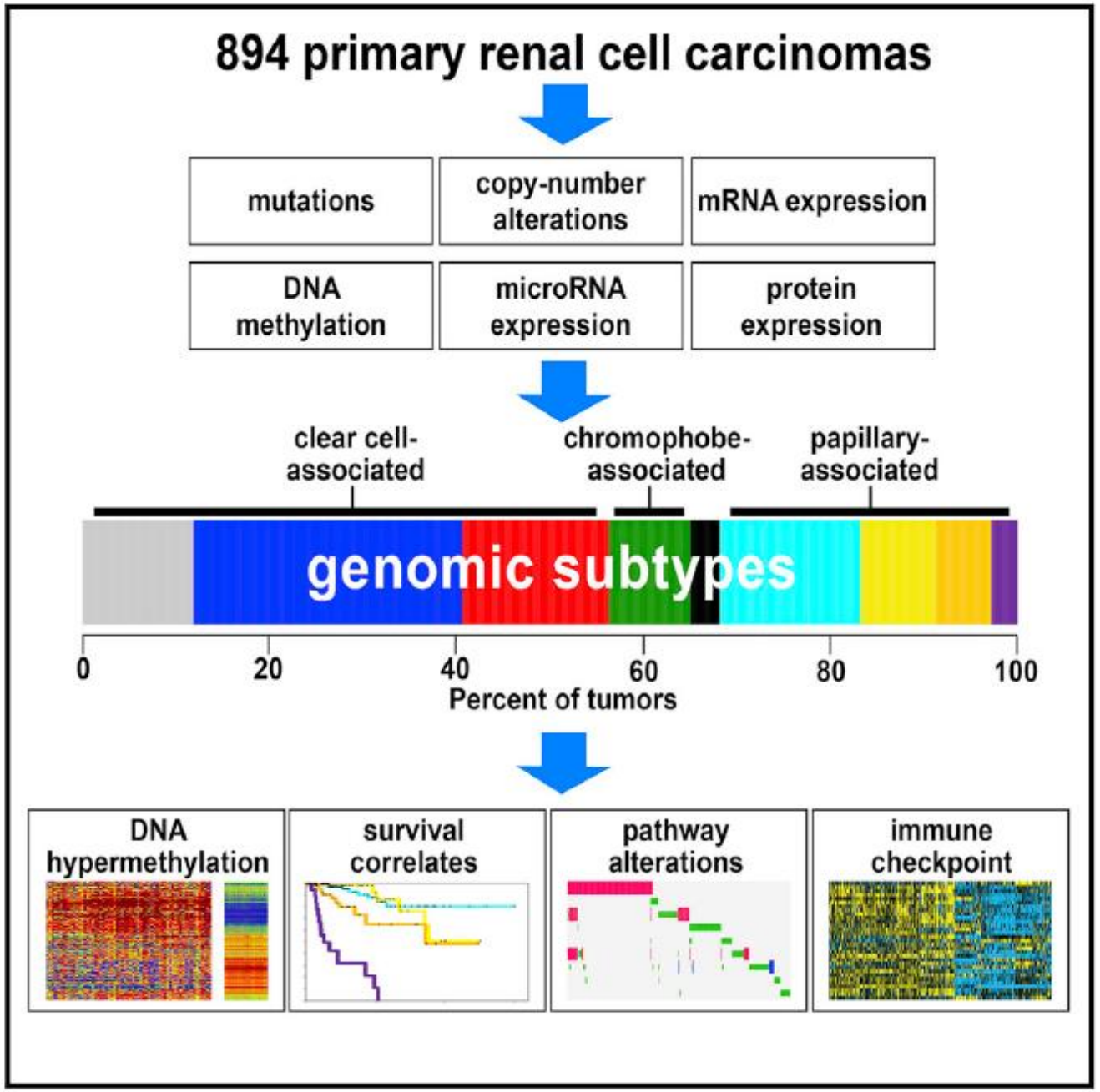
Integrated Multi-omics across RCC subtypes



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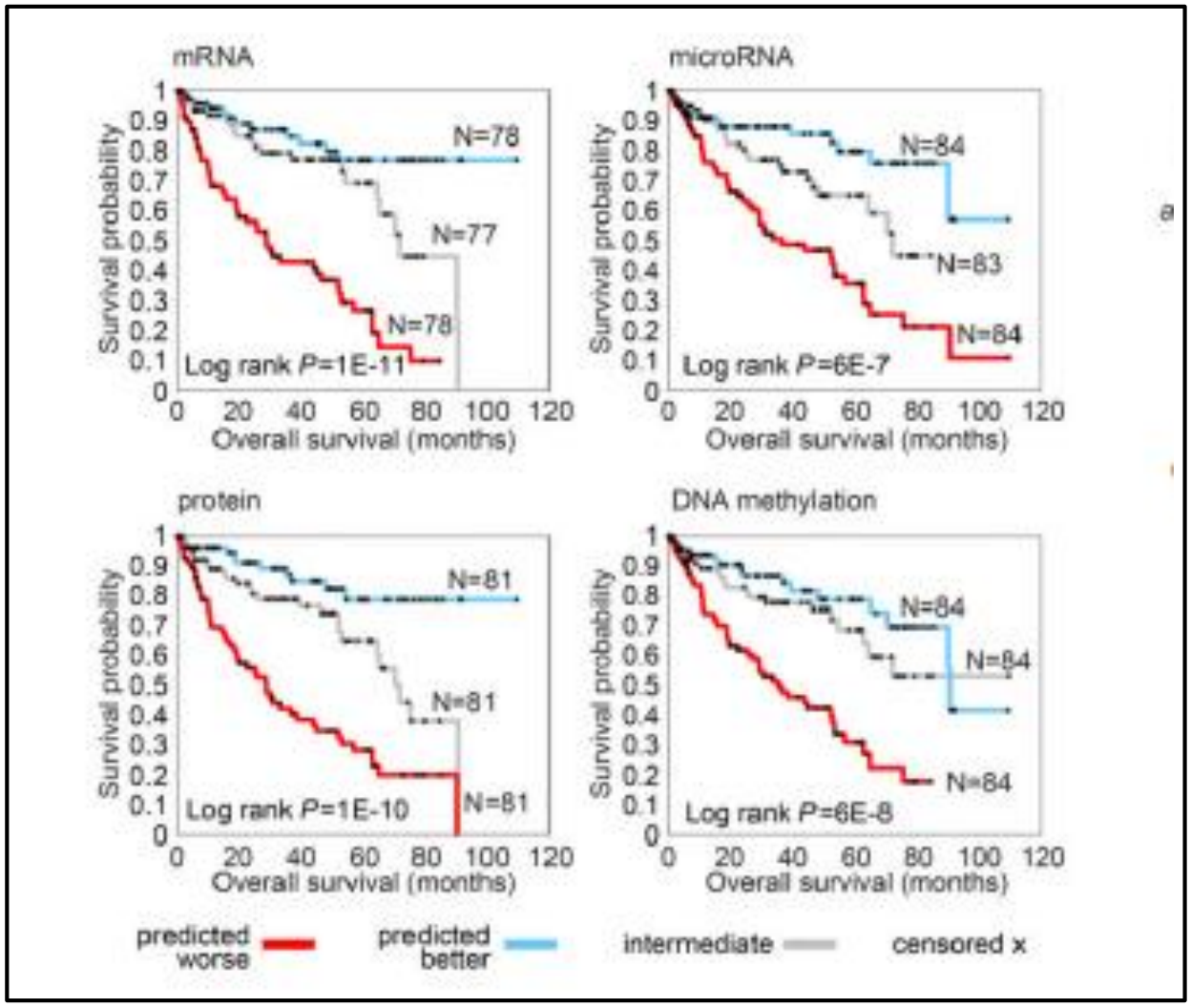

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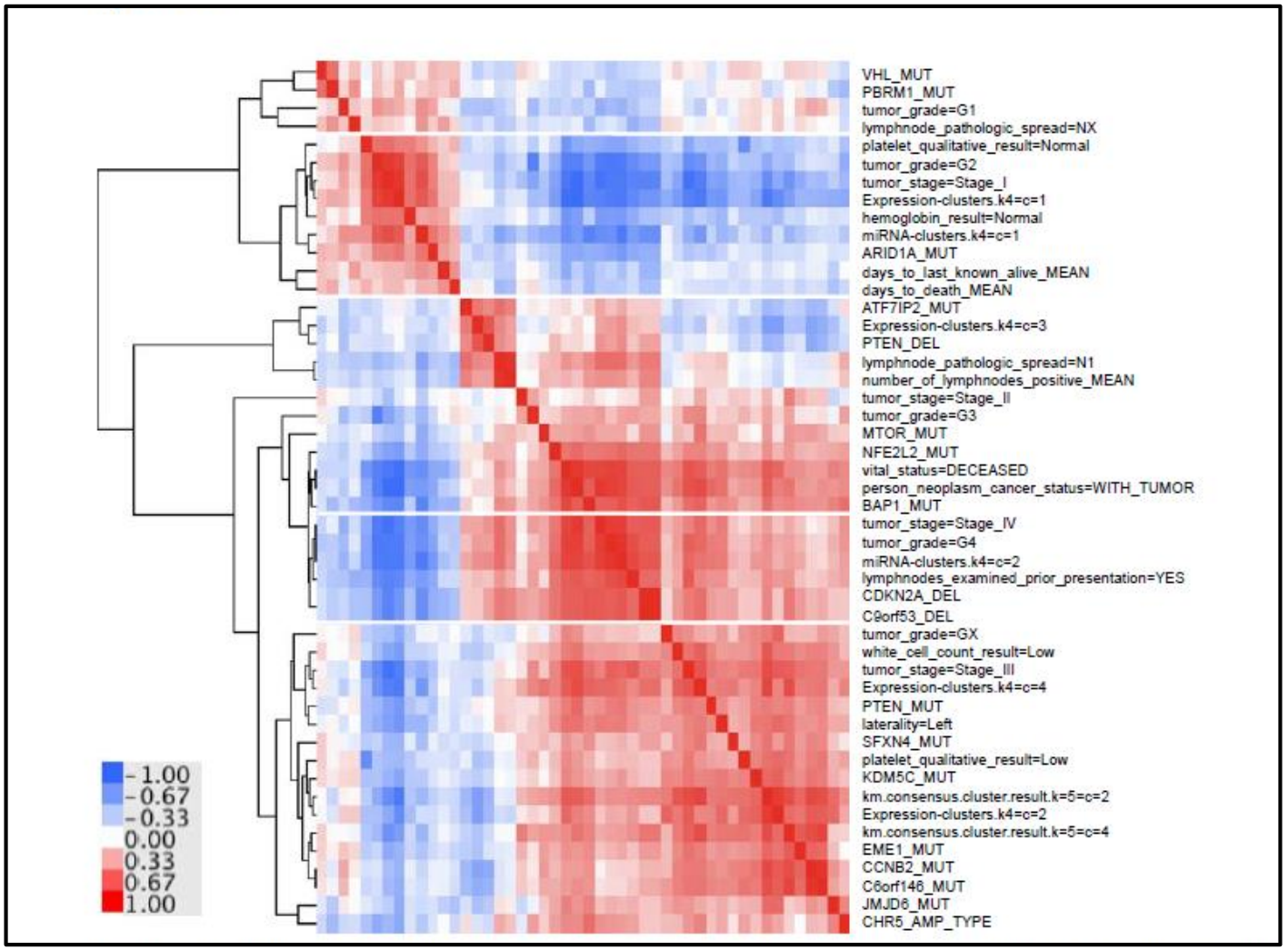

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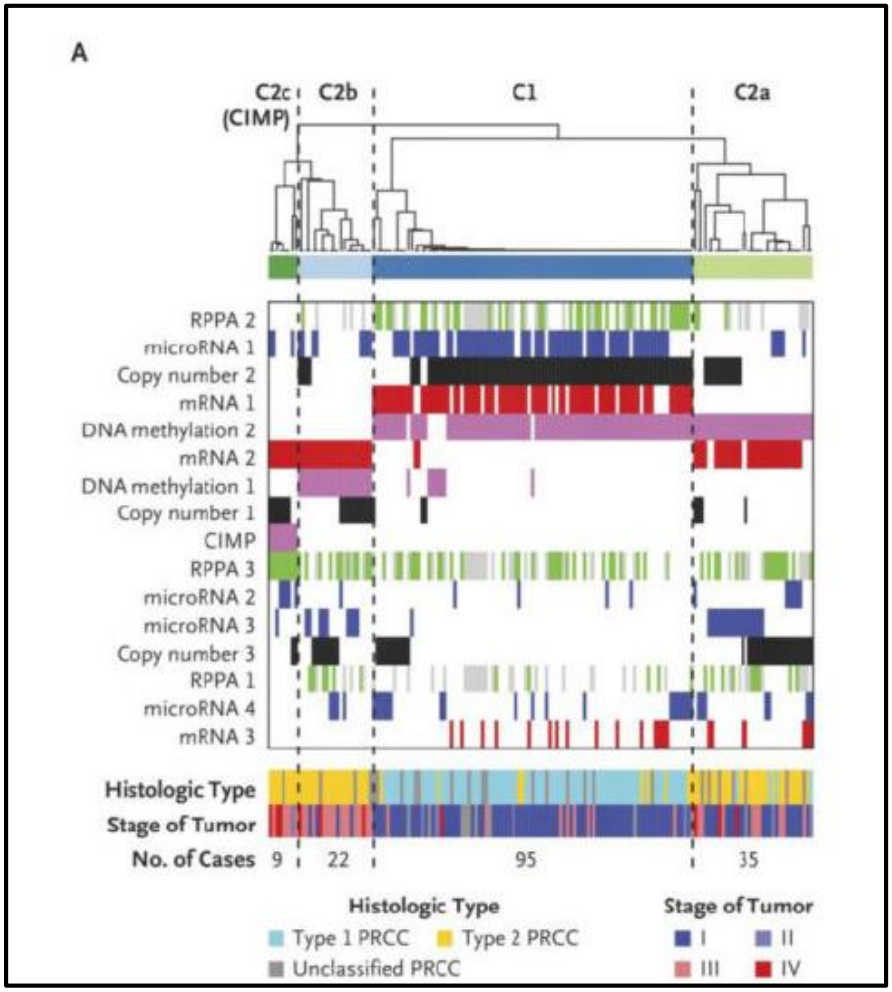

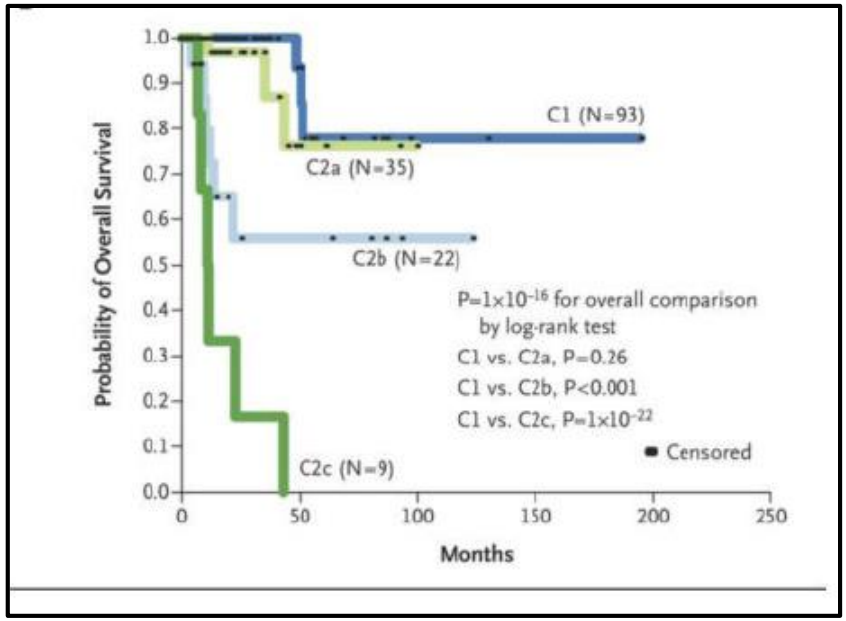

The Cancer Genome Atlas Research Network. Comprehensive Molecular Characterization of Papillary Renal-Cell Carcinoma. N Engl J Med 2016;374:135-45.

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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Immunotherapy Biomarkers

PD-L1/PD-1 Inhibitors

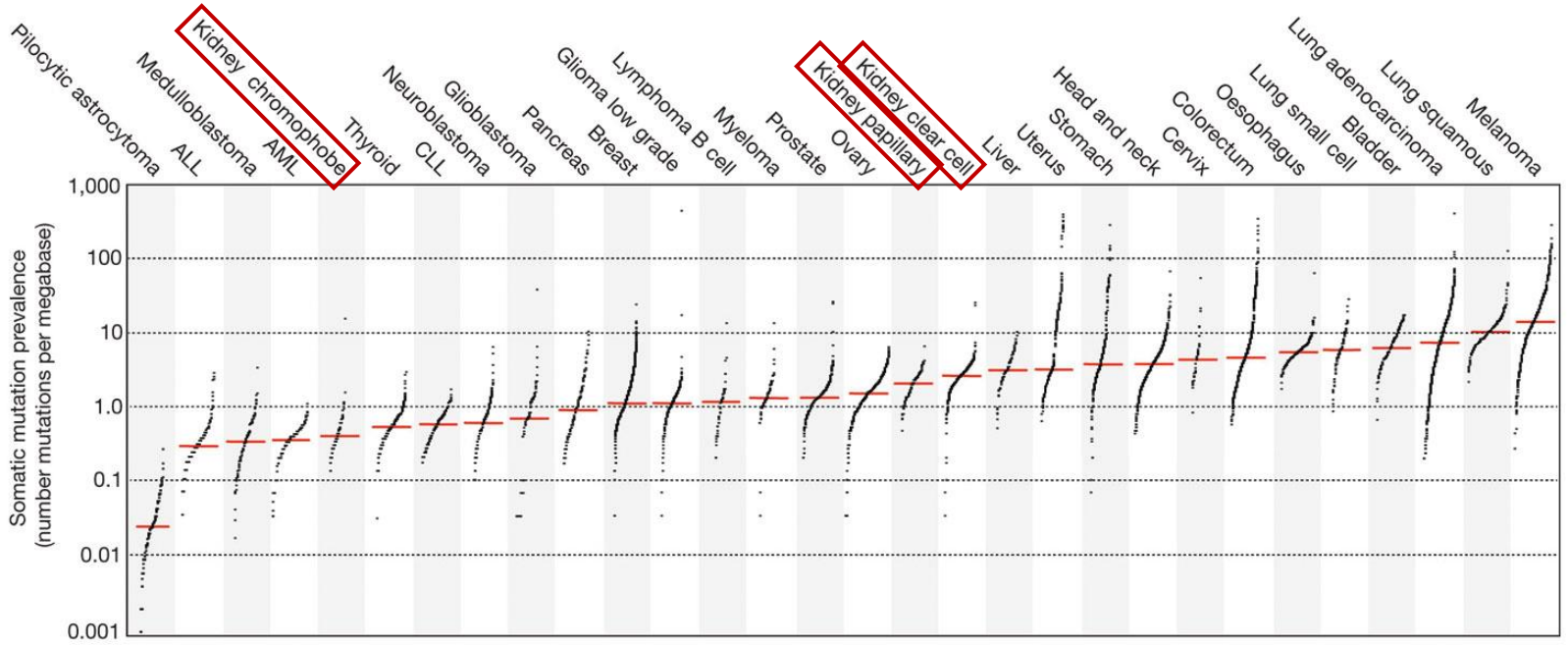
- ccRCC: High expression of several immunotherapy gene targets
  - Greater levels of immune infiltrates
- Many poor risk and Sarcomatoid tumors
  - High levels of PD-L1 expression
  - Greatest relative benefit with nivolumab over everolimus
- CheckMate 025 trial: Higher PD-L1 expression
  - Poor survival
  - 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

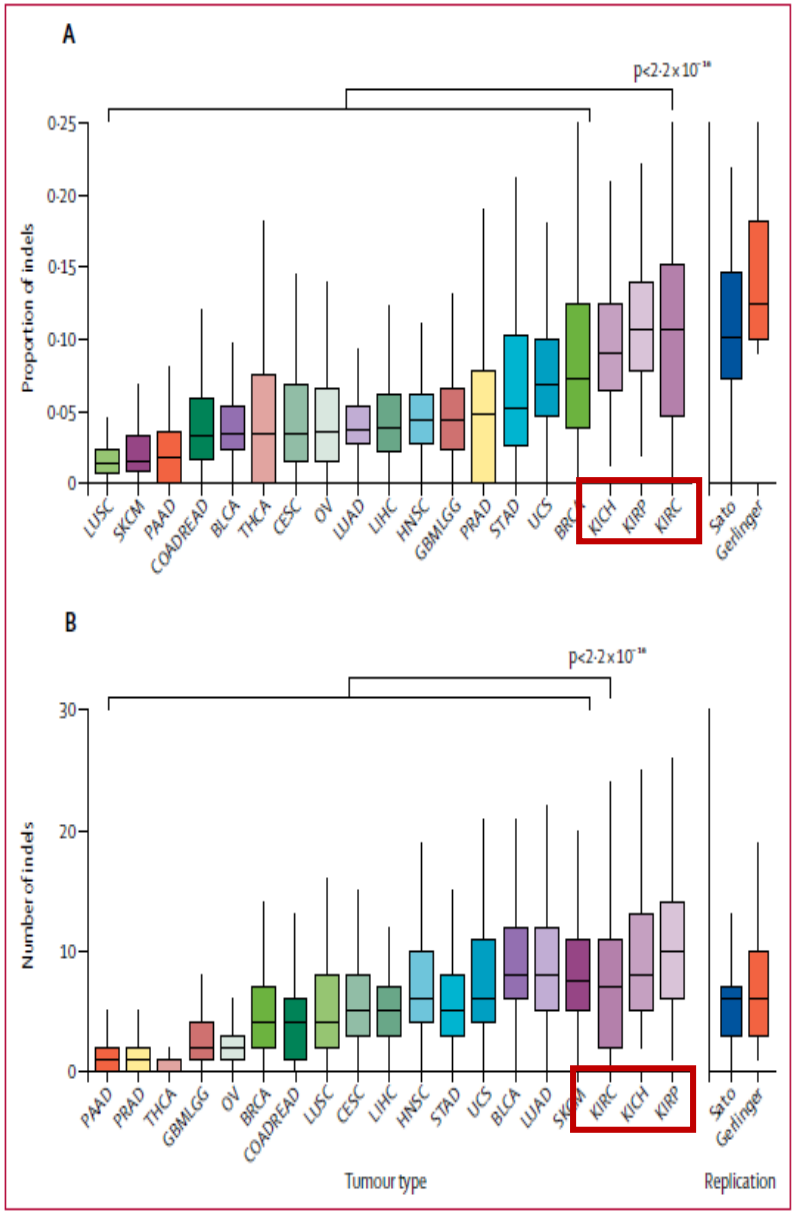
- Different antibodies
- Immune infiltrating cells evaluated
- Intratumoral and intertumoral heterogeneity of PD-L1 expression
- Temporal evolution of PD-L1 status during the development of treatment resistance
- Variation in PD-L1 expression according to the level of tissue hypoxia

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