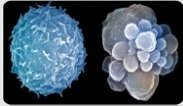# Molecular diagnostics in kidney cancer.
A. Lopez-Beltran (Lisbon, Portugal)

GRAWITZ

Essential alterations that dictate malignant transformation
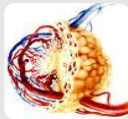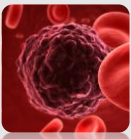
AVOIDING IMMUNE DESTRUCTION

SELF-SUFFICIENCY IN GROWTH SIGNALS

DEREGULATED CELLULAR ENERGETICS

EVADING APOPTOSIS

LIMITLESS REPLICATIVE POTENTIAL

SUSTAINED ANGIOGENESIS

LESS DIFFERENTIATION

TISSUE INVASION & METASTASIS
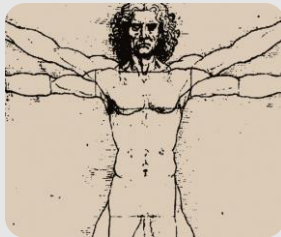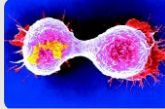
GENOME INSTABILITY AND MUTATIONS
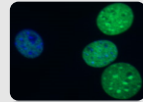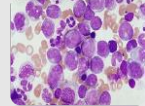
TUMOR PROMOTED INFLAMMATION
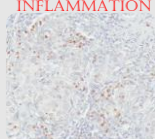
INSENSITIVITY TO ANTIGROWTH SIGNALS

Hanahan and Weinberg, Cell 2011, modified
Hanahan and Weinberg, Cell 2000, modified

1

Molecular diagnostics of Cáncer: A need for improvement
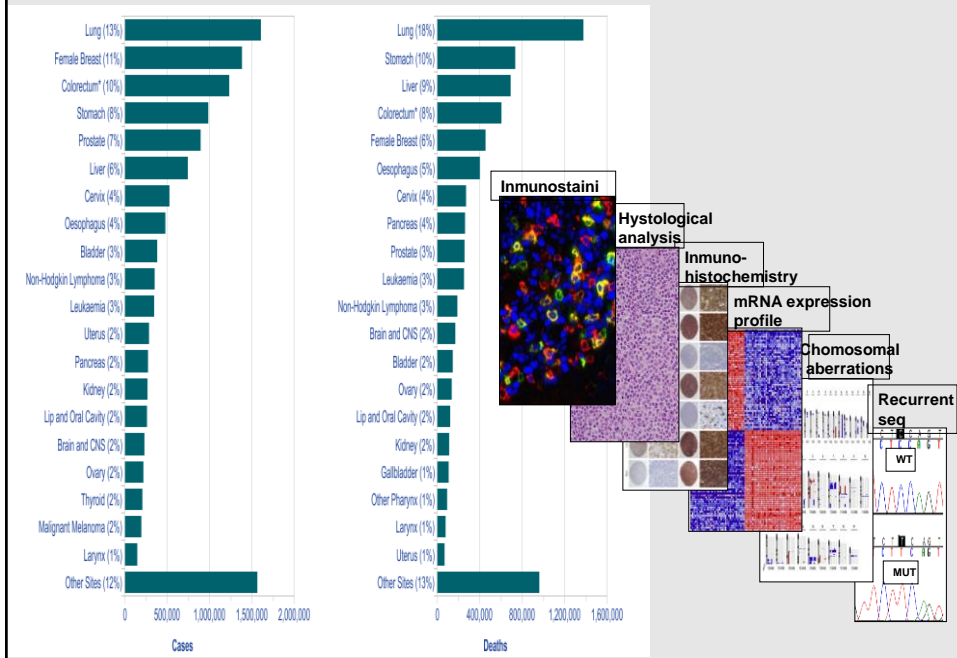
Oncogenes and cancer

Historical Perspective

1911 Peyton Rous, Rockefeller University, NY
Rous Sarcoma Virus,
SRC oncogene-1965 Nobel Prize

"for his discovery of tumour-inducing viruses"

1970 Duesberg and Vogt, RSV 10, ALV 8.5 Kb *vSRC*
1976 Varmus, Bishop, Vogt, Proto-oncogenes
1977 Erickson, Src a kinase
1979 Hunter and Sefton, Src a TK
Growth factor receptors, RTKs
1979 Transfection assays in fibroblasts
(many viral *ONC*)
1982 Weinberg, Cooper, Barbacid, human *RAS*!
1986 Wigler, *MAS* oncogene
1990s GPCRs as ligand-dependent oncogenes
1998-2001 First clinical trial and FDA approval for **Gleevec**
2010s Mutational landscapes of cancer. Cancer consortiums

TIME

THERE IS NEW AMMUNITION IN THE WAR AGAINST CANCER. THESE ARE THE BULLETS.

Revolutionary new pills like GLEEVEC combat cancer by targeting only the diseased cells. Is this the breakthrough we've been waiting for?

Sanger sequencing method
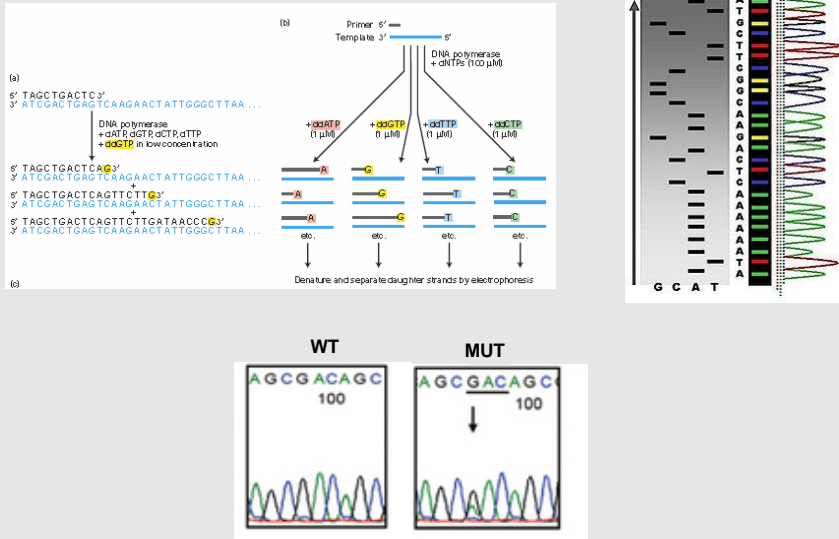
Can detect a range **of 25%-100% mutations** out of the total number of molecules in a sample (tumo[...]
One heterozygous mutation affecting 100% of the tumoral cells constitutes **50% of the mutant** mole[...]

New sequencing based technologies

Figure 1 The workflow of integrating omics data in cancer research and clinical application. NGS technologies detect the genomic, transcriptomic and epigenomic alternations including mutations, copy number variations, structural variants, differentially expressed genes, fusion transcripts, DNA methylation change, etc. Various kinds of bioinformatics tools are used to analyze, integrate, and interpret the data to improve our understanding of cancer biology and develop personalized treatment strategy.

3

Studying the exome with ultrasequencing analysis I

1. Sequencing library:
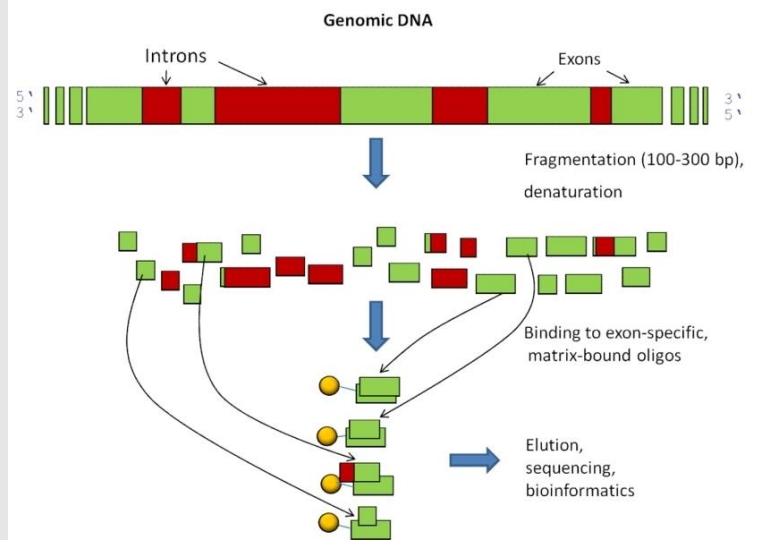
Exome
-20.000 genes
-30 Mb (30×10^6 base pairs):

Studying the exome with ultrasequencing analysis II

Coberture: Percentage of DNA analysed out of the total intended sequencing target
Depth: Number of reads (mean) of each nucleotide: In example 100X

Conclusions

- We need to distinguish between **protein expression and activity.**

- Multiple somatic **mutations can confer tumor resistance** to current targeted therapies at different levels of a signaling pathway

- **Sanger** sequencing allows the detection of mutations when the percentage of mutated DNA molecules is **30%-100%** of the total.

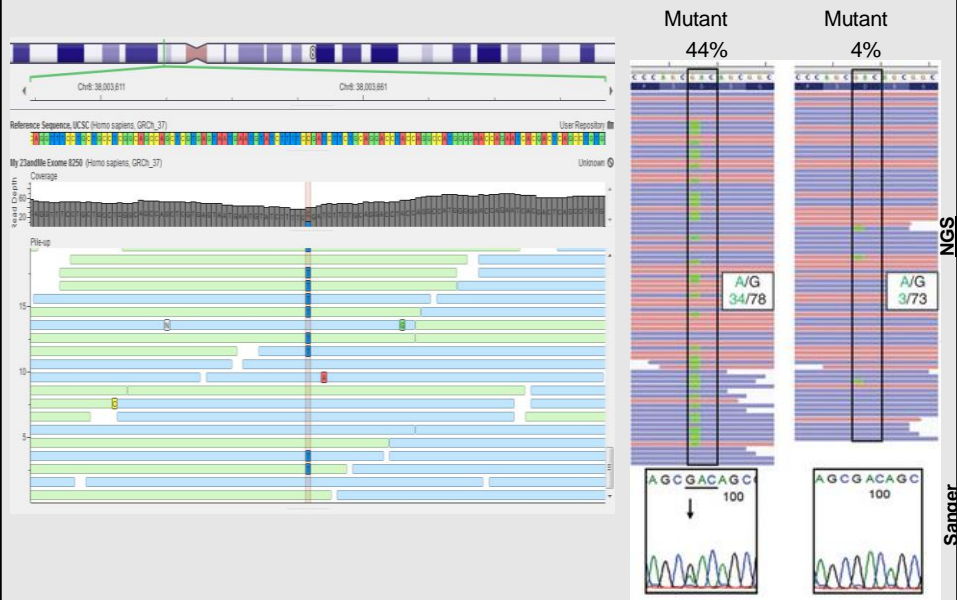- **Next generation sequencing** techniques allows the detection of mutations when the percentage of mutated DNA molecules is as low as **1-4%** of the total, allowing:

      -Studying **microclonal heterogeneity** and dynamics in cancer.
i.e microclones showing resistance to therapy

      -Explore the mutational profile of each cancer towards the identification of **multiple therapeutical targets** simultaneously.

Cáncer diagnostics: A new situation providing rational for our project

- ### Cancer is a multigenic disorder

Therapy targetting mutated genes (BCR-ABL (CML), B-RAF (melanoma) has a lower toxicity, and better efficacy, but still not enough…

- ### High molecular diversity of cancer (A)

Each tumor sample has an unique combination of mutated genes.

Clinical efficacy of targetted therapy needs broad target blockage;

      - Combinatory therapy; i.e MAPK plus PI3K inhibition in hCRC
      - Multitarget therapy; Sorafenib (hCRC).

- ### Tumor dynamics is dominated by (B)

Microclonal competition.

Collaboration stroma-tumor.

Cell Stem Cell
Short Article

Distinct Types of Tumor-Initiating Cells Form Human Colon Cancer Tumors and Metastases

AP Clinical Services Integrated View

Histopathology	Cytopathology
Patient	
Molecular Pathology	Digital Microscopy
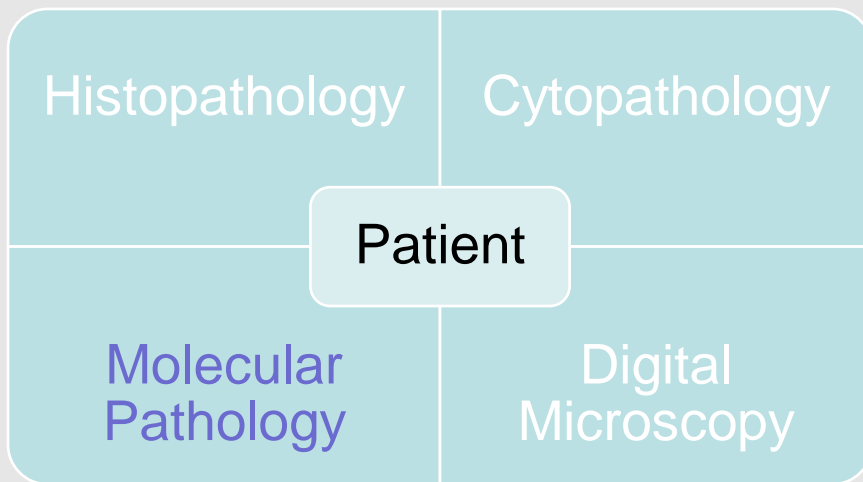
Molecular Pathology or RCC

- **Where we are?**
- **Wher we go?**

Molecular Patology
Diagnostic implications

Mainz Classification of RCC: Histology and Molecular correlation

Thones et al 1968

RCC genetic classification

- Kovacs' genetic classification 1997
- (Heidelberg classification)

Clinical genomics of renal epithelial tumors

Jill M. Hagenkord [a,b], Zoran Gatalica [c], Eric Jonasch [d], Federico A. Monzon [e,f,*]
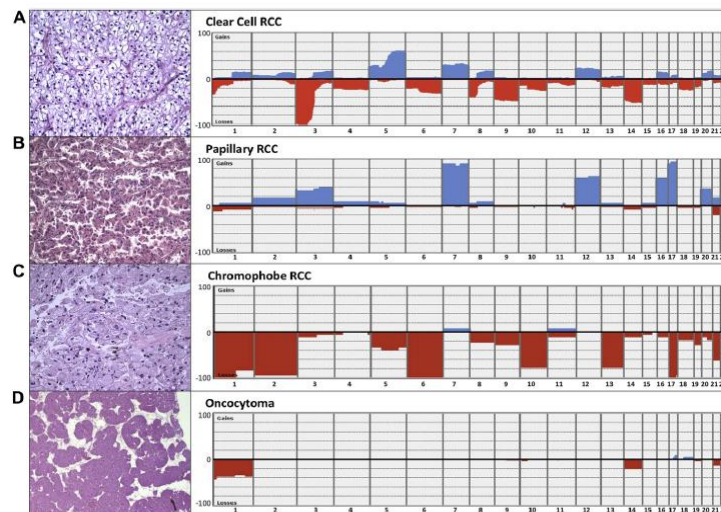
Figure 1   Morphology and genomic profiles for the most common renal epithelial tumors. Each renal epithelial tumor has morphologic (left colum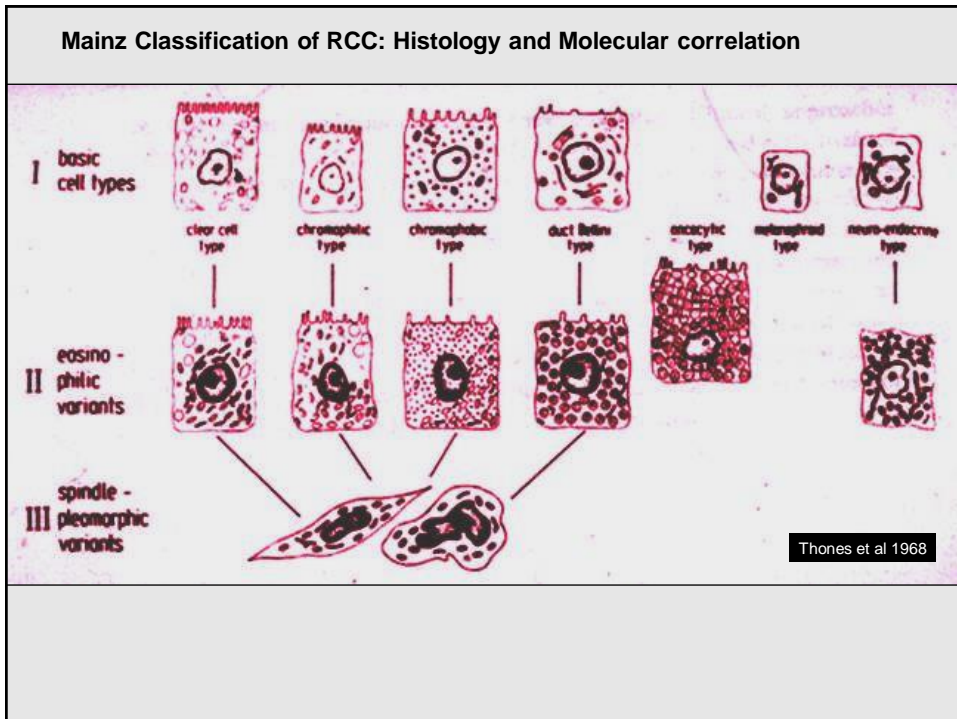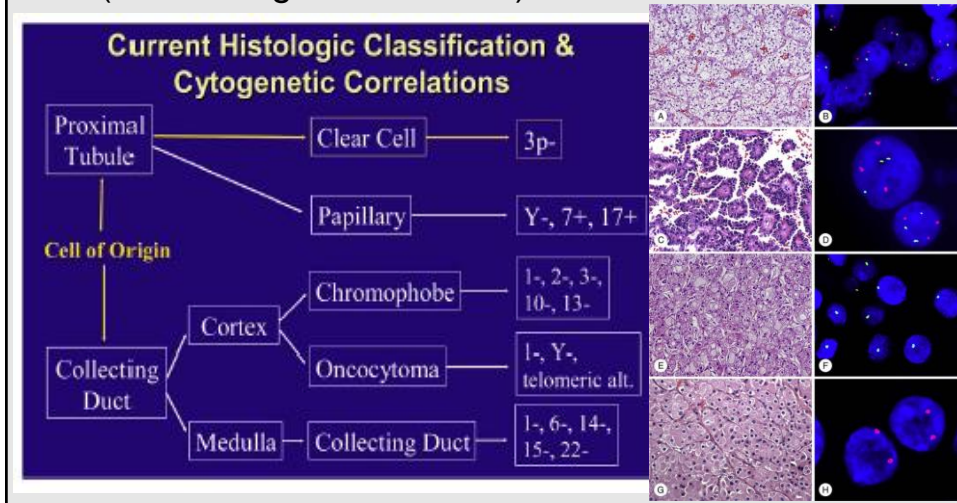n) and chromosomal copy number profiles (right column) that are characteristic to each subtype (red, loss; blue, gain; red stripes, aUPD). (A) Clear cell RCC, n = 130, with characteristic loss of 3p and frequent imbalances in chromosomes 5, 7, 9, and 14. (B) Papillary RCC, n = 26, with characteristic gain of chromosomes 7 and 17 and frequent imbalances in chromosomes 3 (including aUPD), 12, 16, and 20. (C) Chromophobe RCC, n = 18, note hypodiploid complement with frequent losses of chromosomes 1, 2, 6, 10, 13, 17, and 21. (D) Oncocytoma, n = 30, with majority of tumors showing normal chromosomal complement and frequent complete or partial loss of chromosome 1.

Table 1 Frequency of classic chromosomal aberrations in renal epithelial neoplasms

Type of renal tumor	Classic cytogenetic findings	% Cases with chromosomal abnormality	N	Platform	Subtype	Reference
Clear cell RCC	del(3)(p): 3p14, 3p21, 3p25-p26	98	52	LOH		(25)
		98	118	CG		(26)
		81	26	aCGH		(29)
		100	11	FISH		(28)
		100	98	SNP array		(27)
Papillary RCC	Trisomy 7 and/or 17	67/43	19/20	FISH	Low /high grade	(119)
		100/38	9/16	CGH	Type 1/type 2	(120)
		100	6	FISH		(28)
		100/50	19	SNP array	Type 1/type 2	(27)
Chromophobe RCC	Loss of 1, 2, 6, 10, 13, 17 and/or 21	95	10	LOH		(25)
		74	19	FISH		(73)
		100	4	aCGH		(29)
		100	12	SNP array		(27)
Mucinous tubular and spindle cell carcinoma	Loss of 1, 14, and 15	100	6	SNP array		(27)
Oncocytoma	Chr 1 loss or normal	100	10	FISH		(73)
		100	15	SNP array		(27)

Abbreviations: N, number; CG, cytogenetics.

RENAL EPITHELIAL NEOPLASMS; CLINICOPATHOLOGIC FEATURES AND SURVIVAL*

	Clear Cell	Papillary	Chromophobe	Oncocytoma
Cases	410	156	84	97
M : F	1.3:1	2.4:1	1.2:1	1.5:1
Multifocal (%)	9.5	35.2	10.7	14.4
Age	61	60	59	66
Size (cm)	7.1	6.3	8.3	5.1
pT1-pT2 (%)	57	81	70	80
Disease specific survival (5/10yr)	76/70%	86/82%	100/90%	100/100%

*Combined data: AJSP 26;281,2002
Diag Surg Pathol, 4th ed, 2004

Conclusions: Classification schemes for kidney cancer have undergone dramatic changes over the past two decades. Improvements in these classification schemes are important as pathologic variants differ not only in disease biology, but also in clinical behavior, prognosis, and response to systemic therapy. In the era of genomic medicine, further refinements in characterization of RCC subtypes will be critical to the progress of this burgeoning clinical space.
Such, Lopez-Beltran, Martignoni, et al 2014

Clasificacion histológica y genética de los tumores renales, OMS 2004

WHO Classification of Tumours of the Urinary System & Male Genital Organs
IARC, Lyon, 14-18 December 2002

Yellow is Dr Kovacs

WHO histological classifica...

Renal cell tumours
Clear cell renal cell carcinoma
Multilocular clear cell renal cell carcinoma
Papillary renal cell carcinoma
Chromophobe renal cell carcinoma
Carcinoma of the collecting ducts of Bellini
Renal medullary carcinoma
Xp11 translocation carcinomas
Carcinoma associated with neuroblastoma
Mucinous tubular and spindle cell carcinoma
Renal cell carcinoma, unclassified
Papillary adenoma
Oncocytoma

Metanephric tumours
Metanephric adenoma
Metanephric adenofibroma
Metanephric stromal tumour

Leyomiomatous RCC
Thyroid-like RCC
Succinate Dehydrogenase B RCC
Anaplastic lymphoma kinase RCC

TABLE 2. ISUP Vancouver Modification of WHO (2004)
Histologic Classification of Kidney Tumors

Renal cell tumors
  Papillary adenoma
  Oncocytoma
  Clear cell renal cell carcinoma
    Multilocular cystic clear cell renal cell neoplasm of low malignant
      potential*
  Papillary renal cell carcinoma†
  Chromophobe renal cell carcinoma
    Hybrid oncocytic chromophobe tumor*
  Carcinoma of the collecting ducts of Bellini
  Renal medullary carcinoma
  MiT family translocation renal cell carcinoma*
    Xp11 translocation renal cell carcinoma
    t(6;11) renal cell carcinoma*
  Carcinoma associated with neuroblastoma
  Mucinous tubular and spindle cell carcinoma
  Tubulocystic renal cell carcinoma*
  Acquired cystic disease associated renal cell carcinoma*
  Clear cell (tubulo) papillary renal cell carcinoma*
  Hereditary leiomyomatosis renal cell carcinoma syndrome-associated
    renal cell carcinoma*
  Renal cell carcinoma, unclassified
Metanephric tumors
  Metanephric adenoma   Revision of WHO 2004 with expanded categori...
  Metanephric adenofibroma   AJSP, 2014
  Metanephric stromal tumor

10

WHO classification of tumours of the kidney

Renal cell tumours

Clear cell renal cell carcinoma	8310/3
Multilocular cystic renal neoplasm of low malignant potential	8316/1
Papillary renal cell carcinoma	8255/1
Hereditary leiomyomatosis and renal cell carcinoma (HLRCC)-associated renal cell carcinoma	8311/3*
Chromophobe renal cell carcinoma	8317/3
Collecting duct carcinoma	8319/3
Renal medullary carcinoma	8510/3
MiT Family translocation carcinomas	8311/3
Succinate dehydrogenase (SDH)-deficient renal carcinoma	8312/3
Mucinous tubular and spindle cell carcinoma	8480/3
Tubulocystic renal cell carcinoma	8316/3
Acquired cystic disease associated renal cell carcinoma	8316/3
Clear cell papillary renal cell carcinoma	8323/1
Renal cell carcinoma, unclassified	8312/3
Papillary adenoma	8260/0
Oncocytoma	8290/0

WHO 2016

Moleculatr Features in Familiary RCC

Table 1.01 Features of hereditary renal cell tumours

Syndrome	Chromosome(s)	Gene	Protein	Tumour type	Extrarenal manifestations	
					In the dermis	In other organs
Von Hippel–Lindau syndrome	3p25	VHL	Von Hippel–Lindau protein	Multiple, bilateral clear cell renal cell carcinoma; renal cysts		Haemangioblastoma of the retina and central nervous system; phaeochromocytoma; pancreatic and renal cysts; neuroendocrine tumours; epididymal and parametrial cysts; tumours of the inner ear
Hereditary papillary renal cell carcinoma	7p31	MET	MET	Multiple, bilateral papillary renal cell carcinoma (type 1)		
Hereditary leiomyomatosis and renal cell carcinoma	1q42	FH	Fumarate hydratase	Papillary RCC (non–type 1)	Leiomyoma	Uterine leiomyoma/leiomyosarcoma
Familial papillary thyroid carcinoma	1q21	Unknown	Unknown	Papillary renal cell carcinoma, oncocytomas		Papillary thyroid carcinoma
Hyperparathyroidism - jaw tumour syndrome	1q25	HRPT2	Para-fibromin	Mixed epithelial and stromal tumours, papillary renal cell carcinoma		Parathyroid tumours; fibro-osseous jaw tumours
Birt–Hogg–Dubé syndrome	17p11	BHD	Folliculin	Multiple chromophobe renal cell carcinoma, hybrid chromophobe oncocytoma, papillary renal cell carcinoma	Facial fibrofolliculoma	Pulmonary cysts; spontaneous pneumothorax
Tuberous sclerosis	9q34 16p13	TSC1 TSC2	Hamartin Tuberin	Multiple, bilateral angiomyolipomas; lymphangioleiomyomatosis; rare renal cell carcinomas	Angiofibroma, subungual fibroma	Cardiac rhabdomyoma; adenomatous small intestine polyps; pulmonary and renal cysts; cortical tuber; subependymal giant cell astrocytomas
Constitutional chromosome 3 translocations	3p13-14	Unknown	Unknown	Multiple, bilateral clear cell renal cell carcinoma		

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Table 1.02 Features of emerging/provisional renal cell carcinomas

|	Clinical	Morphological	Molecular	Outcome
Oncocytic renal cell carcinoma occurring after neuroblastoma	• Increased incidence of renal cell carcinoma among neuroblastoma survivors • Heterogeneous group, with some MiT family translocation renal cell carcinomas • One distinct oncocytic group with or without exposure to chemotherapy	• Solid, cystic, and papillary • Oncocytic cells with vacuoles and calcification • No distinctive immunohistochemistry	• No molecular marker	• Limited follow-up
Thyroid-like follicular renal cell carcinoma	• Broad age range • Slight female predominance	• Tan-brown gross appearance • Resembles thyroid parenchyma, with follicles and colloid • No distinctive immunohistochemistry, but thyroid transcription factor 1 and thyroglobulin are negative	• Limited studies and no distinctive molecular marker	• Most are indolent • There are rare examples of lymph node and lung metastasis
ALK rearrangement–associated renal cell carcinoma	• Rare (< 10 cases reported) • 3 distinct cases with ALK-vinculin fusion in children with sickle cell trait	For paediatric cases: • Medullary location • Large polygonal/spindle cells • Eosinophilic cytoplasm with intracytoplasmic lumina	• *ALK-VCL* gene fusion	• Limited follow-up
Renal cell carcinoma with (angio)leiomyomatous stroma	• Adults • Male predominance • Historically categorized as a clear cell or clear cell papillary renal cell carcinoma • Has also been called renal angiomyoadenomatous tumour • Occurs sporadically or is associated with tuberous sclerosis	• Branching tubules / papillary tufts • Clear cells • Prominent vascular and smooth muscle stroma • Positive for CK7, 34βE12, and CD10; negative for racemase	• No 3p deletion • No trisomy 7 or 17 • *TCEB1* gene mutation recently described	• Indolent, but limited follow-up

WHO 2016

# MiT family (microphthalmia transcription factor) translocation carcinomas

- Children>>adults>>indolent-to-aggressive

- Chromosome translocation involving the transcription factor E3 (TFE3) located on Xp11.2 and resulting in gene fusions (most commonly with ASPL and PRCC)
  - TFE3 RCC
- Chromosome translocation involving the transcription factor EB (TFEB) on chromosome 6
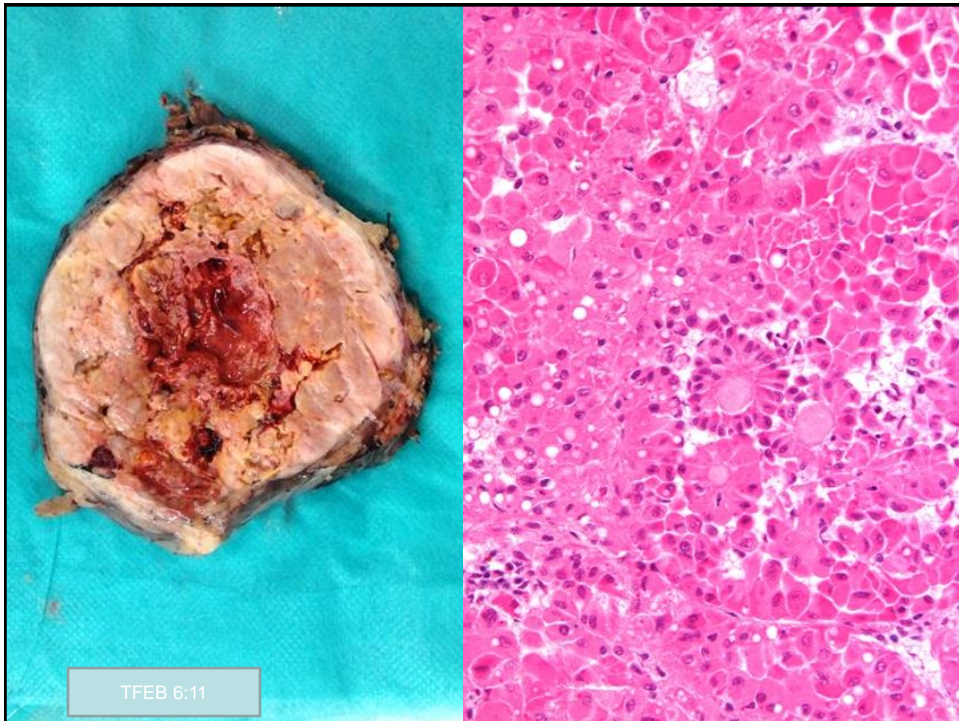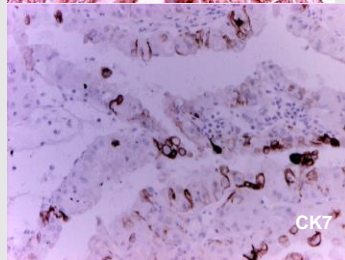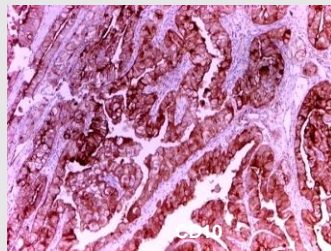 - TFEB RCC

MiTF/TFE translocation carcinomas
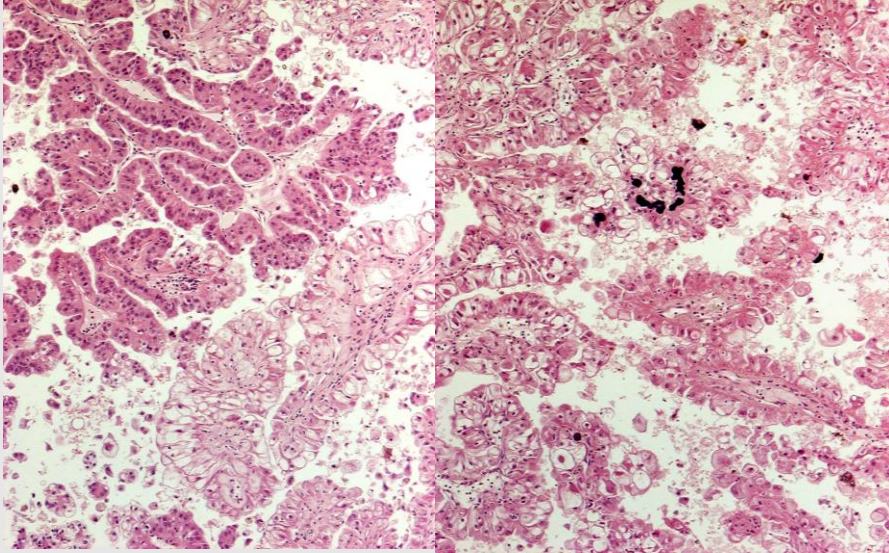
MiTF translocation carcinomas

TTF3

TFEB 6:11
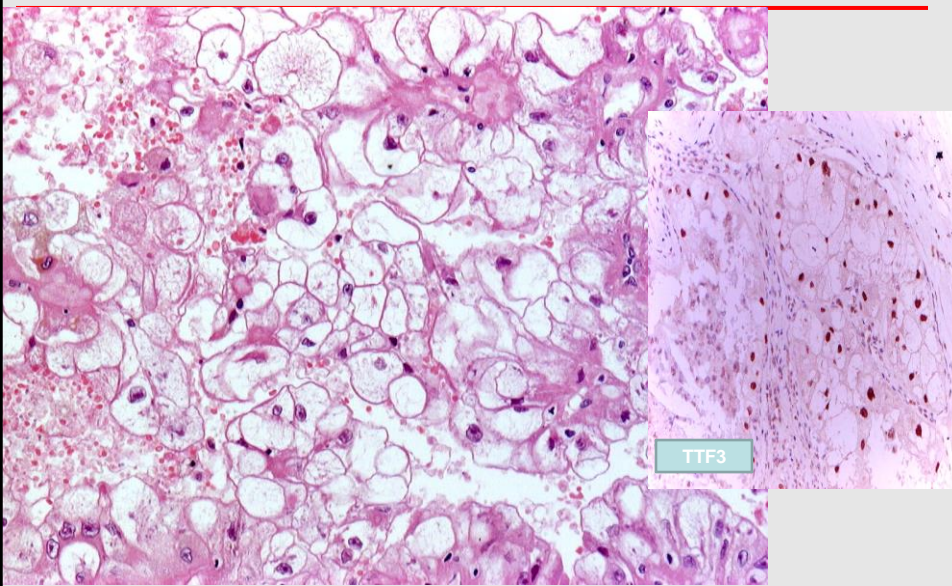
MiTF/TFE translocation carcinomas

- CD10 and racemase positive either diffusely or focally
- EMA, AE1-AE3 and CK7 weakly or focally expressed
- Melan A and HMB45 focally expressed

CD10

CK7

Next-generation sequencing of translocation renal cell carcinoma reveals novel RNA splicing partners and frequent mutations of chromatin remodeling genes

Gabriel G. Malouf[1,#], Xiaoping Su[2,#], Hui Yao[2,#], Jianjun Gao[3], Liangwen Xiong[3], Qiuming

Experimental design—We performed RNA and exome sequencing on an exploratory set of TRCC (n=7), and validated our findings using The Cancer Genome Atlas (TCGA) clear-cell RCC (ccRCC) dataset (n=460).

Results—Using the TCGA dataset, we identified 7 TRCC (1.5%) cases and determined their genomic profile. We discovered three novel partners of *MITF/TFE* (*LUC7L3, KHSRP* and *KHDRBS2*), which are involved in RNA splicing. TRCC displayed a unique gene expression signature as compared to other RCC types, and showed activation of *MITF*, the transforming growth factor β1 and the PI3K complex targets. Genes differentially spliced between TRCC and other RCC types were enriched for *MITF* and *ID2* targets. Exome sequencing of TRCC revealed a distinct mutational spectrum as compared to ccRCC, with frequent mutations in chromatin remodeling genes (six of eight cases, three of which from the TCGA). In two cases, we identified mutations in *INO80D*, an ATP-dependent chromatin remodeling gene, previously shown to control the amplitude of the S phase. Knockdown of *INO80D* decreased cell proliferation in a novel cell line bearing *LUC7L3-TFE3* translocation.

Conclusions—This genome-wide study defines the incidence of TRCC within a ccRCC-directed project and expands the genomic spectrum of TRCC by identifying novel *MITF/TFE* partners involved in RNA splicing and frequent mutations in chromatin remodeling genes.

ALK translocation RCC

ALK-RCC

ALK-RCC
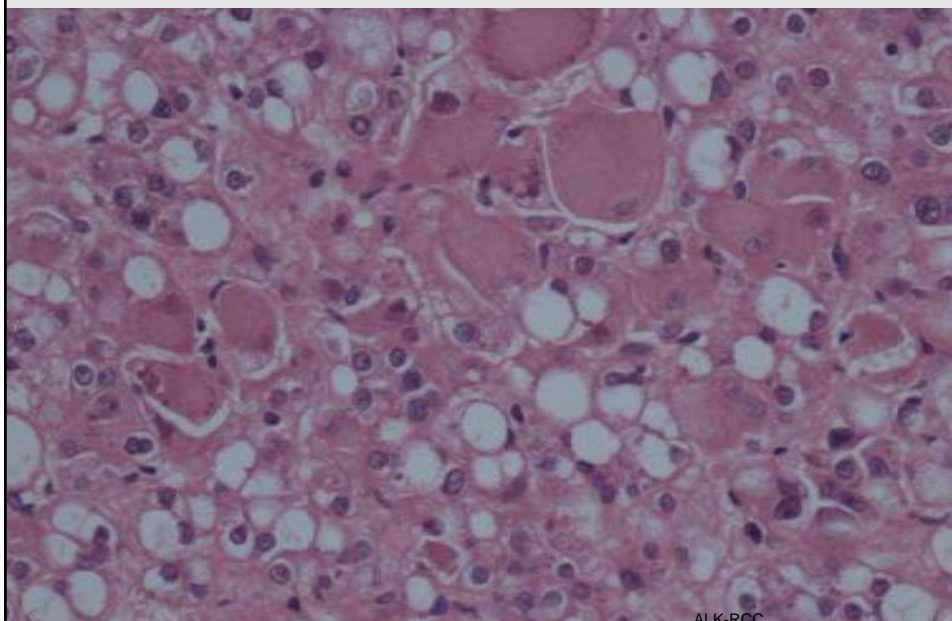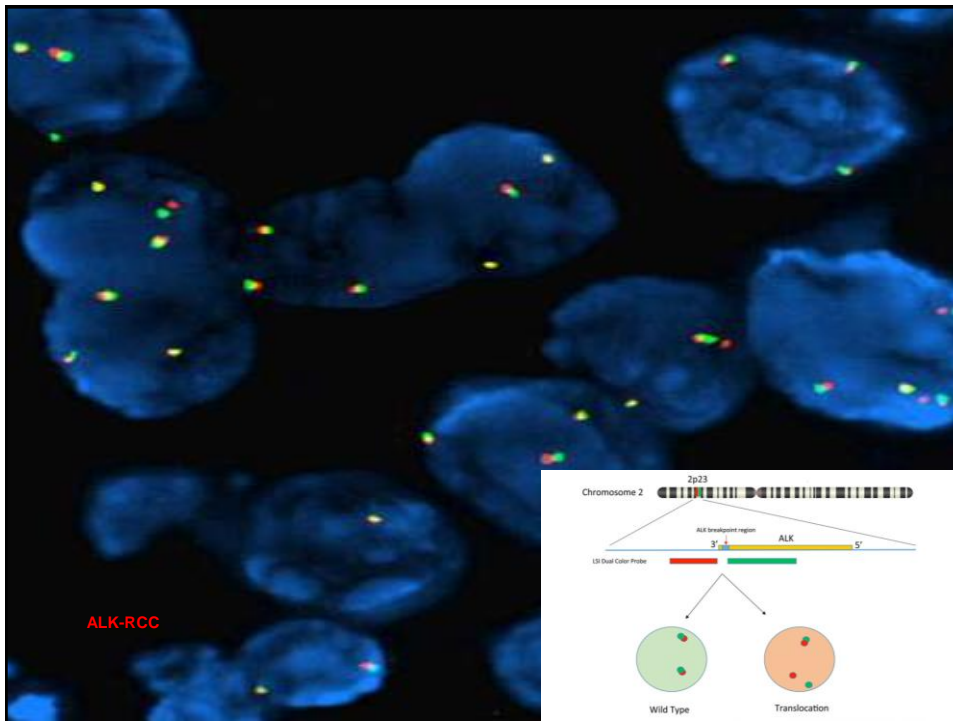
Succinate dehydrogenase-deficient renal cell carcinoma: detailed characterization of 11 tumors defining a unique subtype of renal cell carcinoma

Sean R Williamson[1], John N Eble[2], Mahul B Amin[3], Nilesh S Gupta[1], Steven C Smith[3], Lynette M Sholl[4], Rodolfo Montironi[5], Michelle S Hirsch[4] and Jason L Hornick[4]

Patients with germline mutation of succinate dehydrogenase (SDH) subunit genes are prone to develop paraganglioma, gastrointestinal stromal tumor, and rarely renal cell carcinoma (RCC). However, SDH-deficient RCC is not yet widely recognized. We identified such tumors by distinctive morphology and confirmed absence of immunohistochemical staining for SDHB. Immunohistochemical features were evaluated using a panel of antibodies to renal tumor antigens. Targeted next-generation sequencing was performed on DNA extracted from paraffin-embedded tissue. Eleven tumors were identified from 10 patients, 22–72 years of age (median 40). Two patients had paragangliomas, 1 bilateral SDH-deficient RCC, and 1 contralateral oncocytoma. Grossly, tumors were tan or red–brown, 2–20 cm in diameter (median 4.25 cm). Fuhrman grade was 2 ($n = 10$) or 3 ($n = 1$). Stage was pT1a–pT2b. One patient developed widespread metastases 16 years after nephrectomy and died of disease 6 years later. All tumors were composed of uniform eosinophilic cells containing vacuoles or flocculent cytoplasmic inclusions. Architecture was primarily solid; entrapped renal tubules and intratumoral mast cells were common. By immunohistochemistry, tumor cells were negative for SDHB (11/11) and rarely SDHA (1/11). Labeling was uniformly positive for PAX8 and kidney-specific cadherin and absent for KIT, RCC, and carbonic anhydrase IX. Staining for broad-spectrum epithelial markers was often negative or focal (positive staining for AE1/AE3 in 4/10, CAM5.2 3/7, CK7 1/11, EMA 10/10). By sequencing, *SDHB* mutation and loss of the second allele were present in 5/6 tumors; the SDHA-deficient tumor showed no *SDHB* abnormality. SDH-deficient RCC is a unique neoplasm that is capable of progression, often harboring *SDHB* mutation. A monomorphic oncocytic renal tumor with solid architecture, cytoplasmic inclusions of flocculent material, and intratumoral mast cells should prompt evaluation of SDH status, as it may have implications for screening the patient and relatives. Negative immunohistochemistry for KIT and heterogeneous labeling for epithelial antigens are other supportive features.
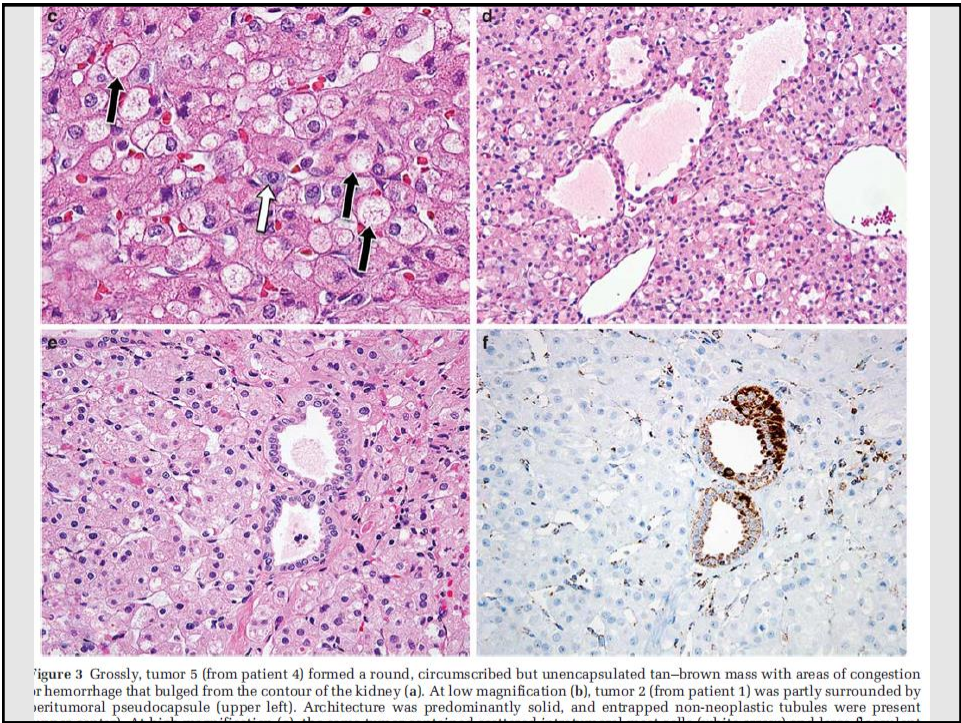
Figure 3 Grossly, tumor 5 (from patient 4) formed a round, circumscribed but unencapsulated tan–brown mass with areas of congestion or hemorrhage that bulged from the contour of the kidney (**a**). At low magnification (**b**), tumor 2 (from patient 1) was partly surrounded by peritumoral pseudocapsule (upper left). Architecture was predominantly solid, and entrapped non-neoplastic tubules were present
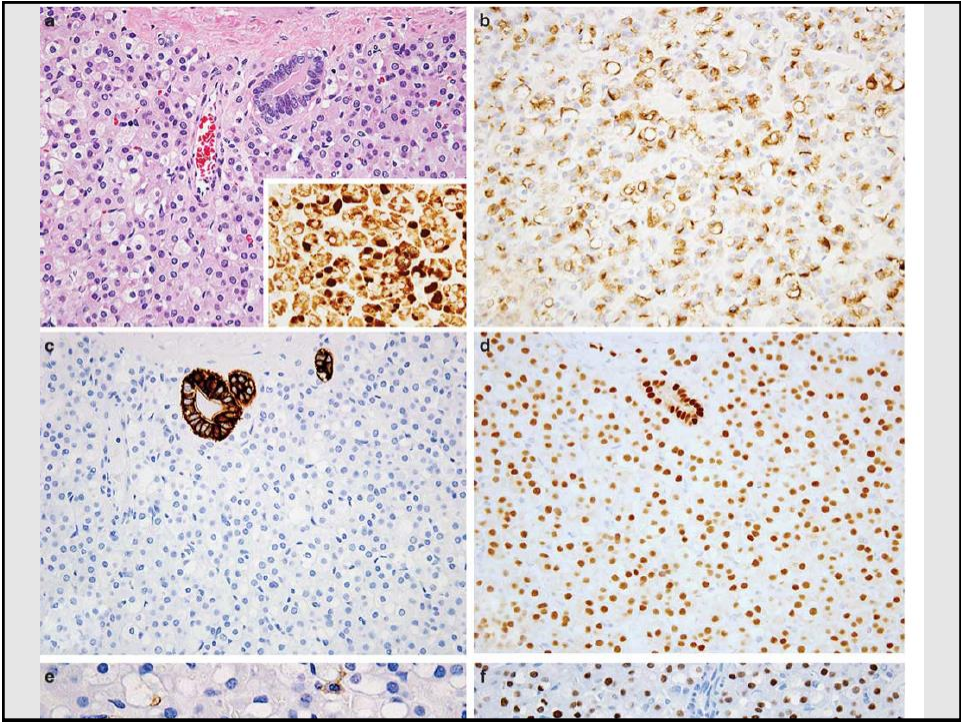
Table 4 *SDHB* gene sequence and copy number alterations detected by targeted next-generation sequencing in SI carcinomas

Tumor	Gene	Sequence-level alteration	AF (%)	% tumor tissue	Copy number alteration
1	*SDHB*	c.137G>A (p.R46Q)	68	90	One copy loss of chr. 1p including *SDHB*
3	*SDHB*	c.859G>A (p.R242H)	85	80	One copy loss of chr. 1p including *SDHB*
5	*SDHB*	c.541-2A>G Splice	62	80	One copy loss of chr. 1p including *SDHB*
8	*SDHB*	c.135C>T (p.R46*)	72	80	One copy loss of chr. 1p including *SDHB*
11	*SDHB*	Exon 3 deletion	NA	95	One copy loss of chr. 1p including *SDHB*

Abbreviations: AF, allelic fraction of sequence alteration; NA, not applicable.
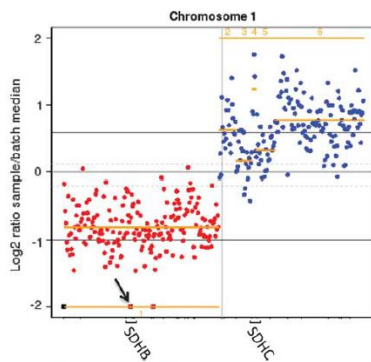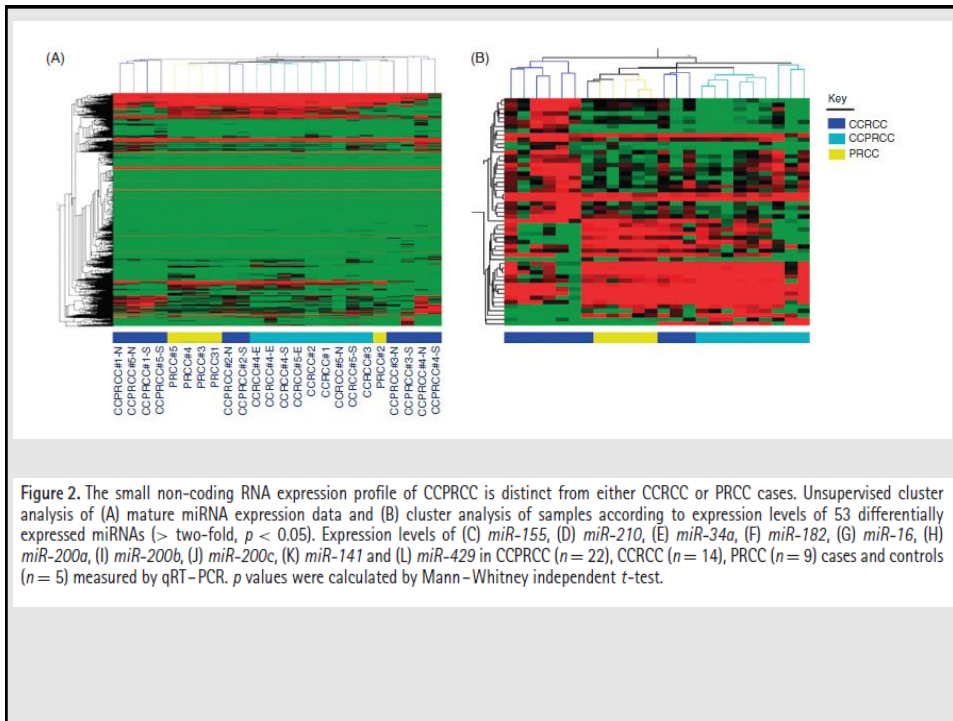

Figure 5 Copy number analysis (one representative sample with SDH deficiency) shows isochromosome 1 with one copy loss of the entirety of 1p and relative gain of 1q. *SDHB*, located on 1p, shows one copy loss across the entire gene and two copy loss at exon 3 (arrow) suggestive of an intragenic deletion or splicing event with subsequent loss of heterozygosity in the tumor. Of note, *SDHC* is located on 1q, and demonstrates no mutational events and low copy gain.

ORIGINAL PAPER

Targeted next-generation sequencing and non-coding RNA expression analysis of clear cell papillary renal cell carcinoma suggests distinct pathological mechanisms from other renal tumour subtypes

Charles H Lawrie,[1,2,3]* Erika Larrea,[1] Gorka Larrinaga,[4] Ibai Goicoechea,[1] María Arestin,[1] Marta Fernandez-Mercado,[1] Ondrej Hes,[5] Francisco Cáceres,[6] Lorea Manterola[1] and José I López[7]

Abstract

Clear cell tubulopapillary renal cell carcinoma (CCPRCC) is a recently described rare renal malignancy that displays characteristic gross, microscopic and immunohistochemical differences from other renal tumour types. However, CCPRCC remains a very poorly understood entity. We therefore sought to elucidate some of the molecular mechanisms involved in this neoplasm by carrying out targeted next-generation sequencing (NGS) to identify associated mutations, and in addition examined the expression of non-coding (nc) RNAs. We identified multiple somatic mutations in CCPRCC cases, including a recurrent [3/14 cases (21%)] non-synonymous T992I mutation in the *MET* proto-oncogene, a gene associated with epithelial-to-mesenchymal transition (EMT). Using a microarray approach, we found that the expression of mature ($n = 1105$) and pre-miRNAs ($n = 1105$), as well as snoRNA and scaRNAs ($n = 2214$), in CCPRCC cases differed from that of clear cell renal cell carcinoma (CCRCC) or papillary renal cell carcinoma (PRCC) tumours. Surprisingly, and unlike other renal tumour subtypes, we found that all five members of the *miR-200* family were over-expressed in CCPRCC cases. As these miRNAs are intimately involved with EMT, we stained CCPRCC cases for E-cadherin, vimentin and β-catenin and found that the tumour cells of all cases were positive for all three markers, a combination rarely reported in other renal tumours that could have diagnostic implications. Taken together with the mutational analysis, these data suggest that EMT in CCPRCC tumour cells is incomplete or blocked, consistent with the indolent clinical course typical of this malignancy. In summary, as well as describing a novel pathological mechanism in renal carcinomas, this study adds to the mounting evidence that CCPRCC should be formally considered a distinct entity. Microarray data have been deposited in the GEO database [GEO accession number (GSE51554)].

Figure 2. The small non-coding RNA expression profile of CCPRCC is distinct from either CCRCC or PRCC cases. Unsupervised cluster analysis of (A) mature miRNA expression data and (B) cluster analysis of samples according to expression levels of 53 differentially expressed miRNAs (> two-fold, $p < 0.05$). Expression levels of (C) *miR-155*, (D) *miR-210*, (E) *miR-34a*, (F) *miR-182*, (G) *miR-16*, (H) *miR-200a*, (I) *miR-200b*, (J) *miR-200c*, (K) *miR-141* and (L) *miR-429* in CCPRCC ($n = 22$), CCRCC ($n = 14$), PRCC ($n = 9$) cases and controls ($n = 5$) measured by qRT–PCR. p values were calculated by Mann–Whitney independent t-test.

Genetic mutations in accordance with a low malignant potential tumour are not demonstrated in clear cell papillary renal cell carcinoma

Maria Rosaria Raspollini,[1] Francesca Castiglione,[1] Liang Cheng,[2] Rodolfo Montironi,[3] Antonio Lopez-Beltran[4,5]

ABSTRACT

Clear cell papillary renal cell carcinoma (CCPRCC) cases were evaluated for mutations on the following genes: KRAS, NRAS, BRAF, PIK3CA, ALK, ERBB2, DDR2, MAP2K1, RET and EGFR. Four male and three female patients of age 42–74 years were evaluated. All cases were incidentally detected by ultrasound and ranged 1.8–3.5 cm. Microscopic examination showed variably tubulopapillary, tubular acinar, cystic architecture and the characteristic linear arrangement of nuclei. The cells were reactive with CK7 (strong), CA IX (cup-shape) and 34 β E12. CD10, AMACR/RACEMASE and GATA3 were negative. There were no mutations on any of the investigated genes. This preliminary observation supports the concept that CCPRCC might be indeed an indolent tumour worth it to be named as clear cell papillary neoplasm of low potential.

Table 1 Genes and codons evaluated in the study

Gene	Codon
KRAS	12
KRAS	13
KRAS	18
KRAS	59
KRAS	61
KRAS	117
KRAS	146
NRAS	12
NRAS	13
NRAS	18
NRAS	59
NRAS	61
NRAS	117
NRAS	146
BRAF	11
BRAF	15
PIK3CA	9
PIK3CA	20
ALK	22
ALK	23
ALK	25
ERBB2	20
DDR2	9
DDR2	16
DDR2	18
MAP2K1	2
RET	16
EGFR	18
EGFR	19
EGFR	20

Characterization of Clinical Cases of Collecting Duct Carcinoma of the Kidney Assessed by Comprehensive Genomic Profiling

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Background: Collecting duct carcinoma (CDC) is a rare type of renal cell carcinoma (RCC) originating from the renal medulla. Clinical outcomes are poor, and there are no consensus guidelines to guide therapy.

Objective: To determine genomic alterations (GAs) in a series of patients with locally advanced or metastatic CDC for whom genomic profiling was performed during the course of clinical care.

Design, setting, and participants: Formalin-fixed, paraffin-embedded blocks or slides were obtained for 17 patients with CDC. DNA was extracted and comprehensive genomic profiling was performed in a laboratory certified under the Clinical Laboratory Improvement Amendments.

Outcome measurements and statistical analysis: Bayesian algorithms and local alignment algorithms were used to detect substitutions and insertions/deletions, respectively. A comparison to normal control samples was used to detect copy number alterations. Clinically relevant GAs (CRGAs) were defined as those linked to approved or investigational targeted therapies.

Results and limitations: The median age in the cohort was 53 yr (range 26–73), and 14 primary tumors and three metastatic sites assessed. A total of 36 GAs were detected in this series of patients, with an average of 2.1 GAs per case. The most common GAs were in *NF2* (5/17, 29%), *SETD2* (4/17, 24%), *SMARCB1* (3/17, 18%), and *CDKN2A* (2/17, 12%). Of nine cases assessed for *FH* GAs, two patients had *FH* homozygous loss. A limitation is that targeted interrogation of genes known to be implicated in other cancers was performed, so mutations outside of these cannot be excluded.

Conclusions: Recurrent CRGAs were detected in this series of CDC cases and suggest a possible benefit from targeted therapy. In particular, mTOR inhibitors may be of interest in patients with *NF2* alterations. Alterations in *FH* and *SMARCB1* also occurred in a mutually exclusive manner to *NF2* alterations.

Patient summary: This report provides important genomic insights into collecting duct carcinoma, a rare type of renal cell carcinoma with a very aggressive course. These insights could further rationalize the use of targeted therapies for rare tumors according to the individual genomic alterations harbored.
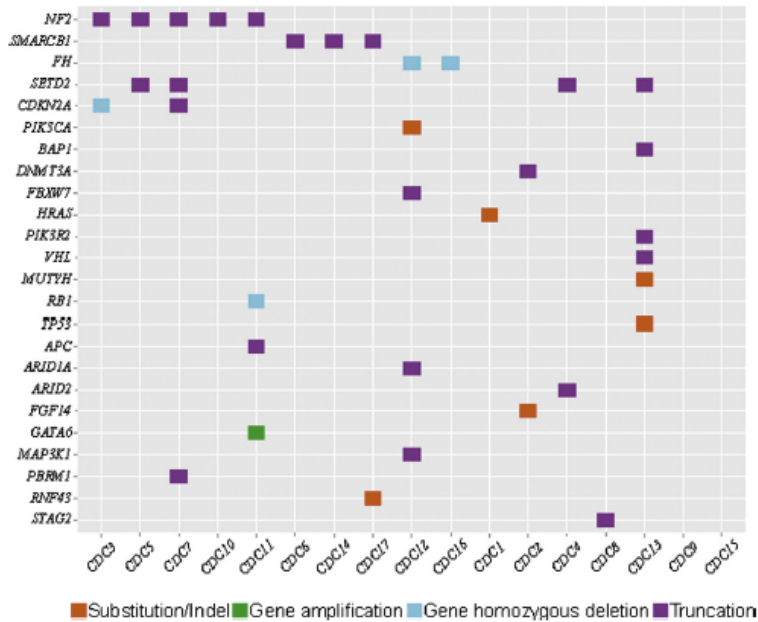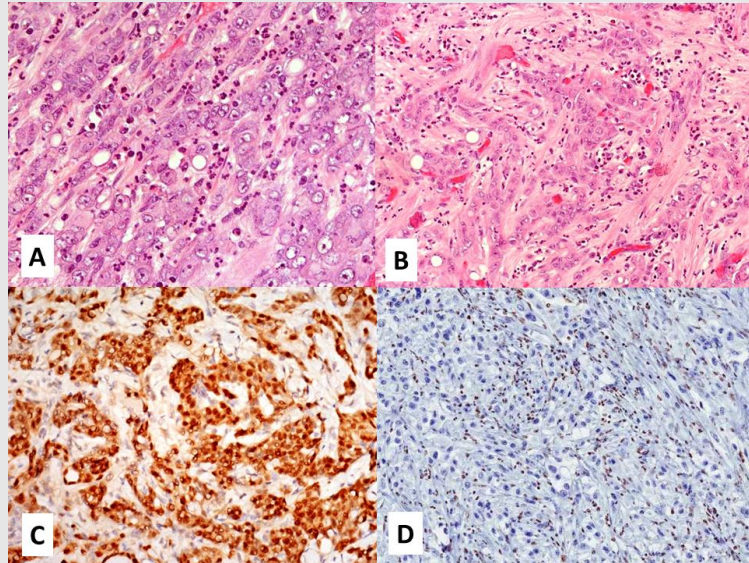
Fig. 1 – Tile plot of genomic alterations observed in 17 cases of collecting duct carcinoma and renal medullary carcinoma.
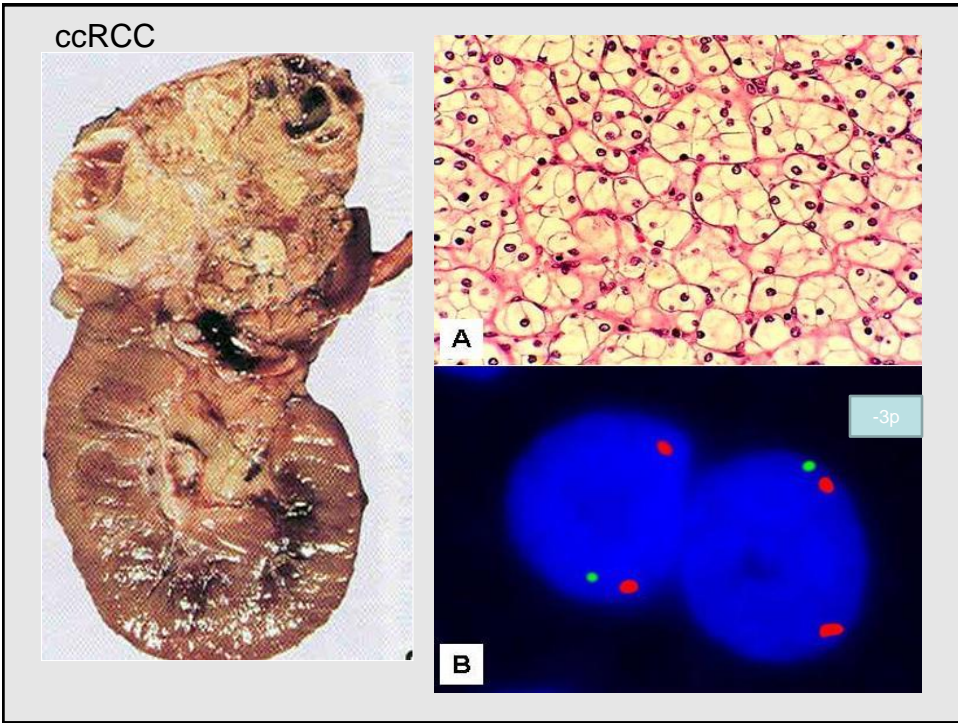
SMARCB1/INI1 Genetic Alterations in Renal Medullary Carcinomas

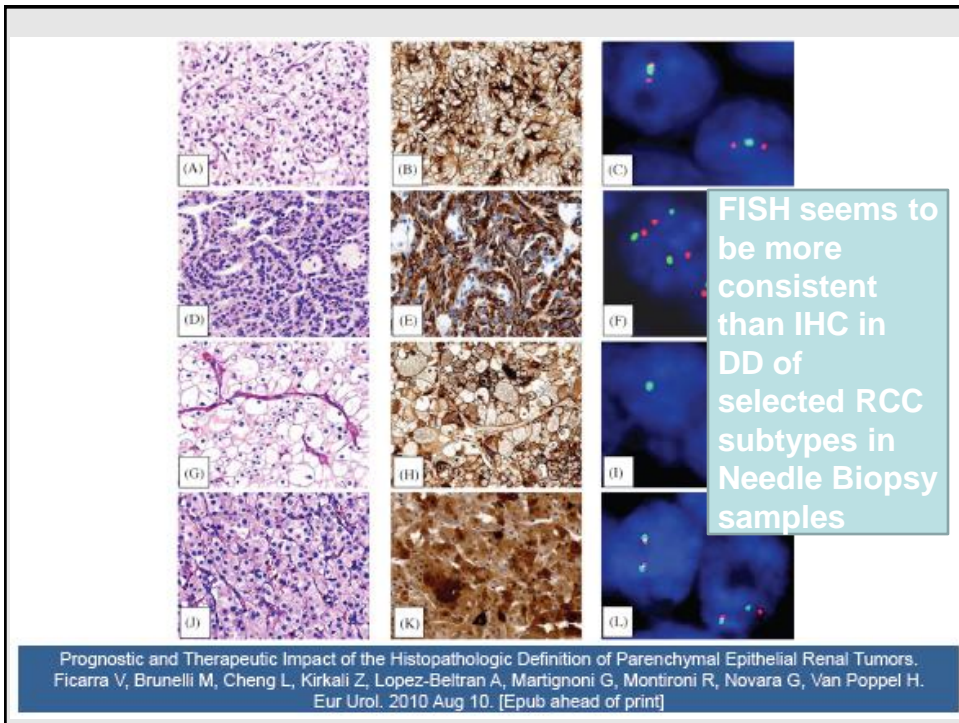Antonio Lopez-Beltran [a,b,*], Liang Cheng [c], Maria R. Raspollini [d], Rodolfo Montironi [e]

Eur Urol 2016

Molecular Pathology and ommon subtypes of RCC

ccRCC

A

B

-3p

QUALITATIVE ANALYSIS OF IHC MARKERS IN RENAL TUMORS

	Clear cell RCC	Papillary RCC	Chromophobe RCC	Oncocytoma
Vimentin	Cytoplasmic Diffuse	Absent*	Absent*	Absent
CA IX	Membranous Cytoplasmic Diffuse	Cytoplasmic Focal Tips/necrosis	Cytoplasmic Focal (rare)	Absent
CD10	Membranous Cytoplasmic Diffuse	Membranous/ apical Focal or diffuse	Cytoplasmic Focal	Cytoplasmic Focal
AMACR	Cytoplasmic Focal or diffuse	Cytoplasmic Finely granular Diffuse	Cytoplasmic Focal	Cytoplasmic Focal
CK7	Cytoplasmic Focal	Membranous Diffuse	Membranous Diffuse	Cytoplasmic Focal (rare)
CD117	Cytoplasmic Focal	Cytoplasmic Focal (rare)	Membranous Diffuse	Cytoplasmic Diffuse

FISH seems to be more consistent than IHC in DD of selected RCC subtypes in Needle Biopsy samples

Prognostic and Therapeutic Impact of the Histopathologic Definition of Parenchymal Epithelial Renal Tumors. Ficarra V, Brunelli M, Cheng L, Kirkali Z, Lopez-Beltran A, Martignoni G, Montironi R, Novara G, Van Poppel H. Eur Urol. 2010 Aug 10. [Epub ahead of print]

Biomarquers and CS mostality

Table 2 – Postoperative assessment of cancer-specific mortality

Model	Sample size	Target population	Predictors	C-index
Zisman et al. [14]	661	RCC of all stages	- AJCC - Fuhrman grade - ECOG-PS	82–86%
Zisman et al. [15]	814	RCC of all stages	- TNM (1997) plus ECOG-PS	73% 79–86%
Frank et al. [30]	1801	Localized clear cell RCC	- TNM (1997) - Tumour size - Nuclear grade - Tumour necrosis	85% (int.) 81–82% (val.)
Kim et al. [86]	318	RCC of all stages	- M stage - Metastatic CAIX - p53 - Vimentin - Gelsolin	79%
Kim et al. [187]	150	Metastatic clear cell RCC	- T stage - ECOG-PS - CAIX - Vimentin - p53 - PTEN	68%
Thompson et al. [116]	1560	Localized clear cell RCC	- TNM (1997) - Tumour size - Nuclear grade - Tumour necrosis	n.r.
Karakiewicz et al. [23]	2530 (dev.) 1422 (val.)	Clear cell, papillary, chromophobe RCC	- pT stage - pN stage - M stage - Tumour size - Fuhrman grade - Symptoms classification	88–89% (val.)
Karakiewicz et al. [189]	2530 (dev.) 3560 (val.)	RCC of all stages	- pT stage - pN stage - M stage - Tumour size - Fuhrman grade - Symptoms classification	87–91% (val.)
Parker et al. [29]	818	Clear cell RCC	- B7-H1 - Survivin - Ki-67	73%

RCC = renal cell carcinoma; AJCC = American Joint Committee on Cancer; ECOG-PS = Eastern Cooperative Oncology Group Performance Status; int. = internal; val. = validation; CAIX = carbonic anhydrase IX; PHEN = phosphatase and tensin homolog; dev. = development.

Table 5 – Molecular marker and its association with other established clinical and/or pathological characteristics of renal cell carcinoma (RCC)

Marker	Histology	Tumor stage	Tumor size	Tumor necrosis	Tumor grade	Metastatic disease/progression	Other
Neutrophil							
C-reactive protein						+	
VHL						+	
HIF-α	+(clear cell)						
Tissue-based							
VEGF	+/ns (papillary)	+	+	+	+		Predictor of microvessel invasion
CAIX				ns	ns	+	
mTOR							
pS6						+	
PTEN		+					
pAkt					+		+
Other							
Caveolin-1	+(clear cell)						
p53	+(papillary)				+		
Ki-67							
Survivin	+(all HS)				+		Predictor of aggressive disease
B7-H1						+	
Vimentin	+(clear cell/papillary)						
Fascin	+(sarcomatoid)	+	+			+	
MMP	+(non clear cell)				+		Predictor of aggressive disease
IMP3	+(sarcomatoid)	+		+		+	Predictor of lymph node involvement
Blood-based							
VEGF							
CAIX	+(clear cell)	+/ns	+		ns		
NGAL							
SAA						+	
IGF-I							
NMP-22							Predictor of RCC diagnosis

+: associated with; ns: not significant.

Genetic Clustering of Clear Cell Renal Cell Carcinoma Based on Array-Comparative Genomic Hybridization: Its Association with DNA Methylation Alteration and Patient Outcome

Eri Arai,[1] Saori Ushijima,[1] Hitoshi Tsuda,[6] Hiroyuki Fujimoto,[4] Fumie Hosoda,[2] Tatsuhiro Shibata,[2] Tadashi Kondo,[3] Issei Imoto,[5] Johji Inazawa,[5] Setsuo Hirohashi,[1] and Yae Kanai[1]

BOTH CLUSTERS	Loss of chromosome 3p and gain of 5q and 7
CLUSTER B	Loss of 1p, 4, 9,13q, and14q
CLUSTER B	High number of methylated CpGislands
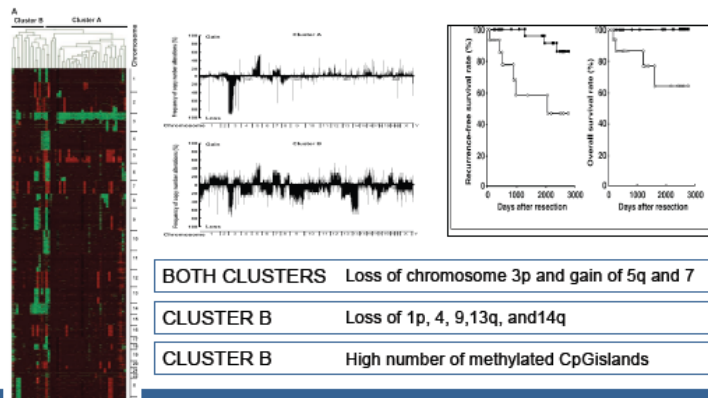
Clin Cancer Res. 2008 1;14:5531-9.

Genome-Wide Promoter Methylome of Small Renal Masses
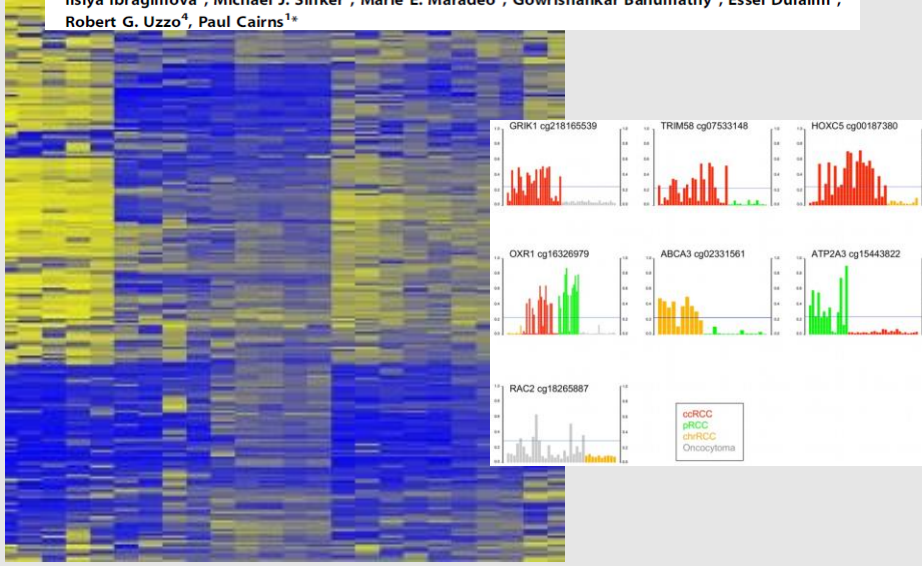
Loss of chromosome 9p is an independent prognostic factor in patients with clear cell renal cell carcinoma

Mod Pathol. 2008;21:1-6

Points 0 10 20 30 40 50 60 70 80 90 100

T stage

N stage

M stage

Fuhrman grade

Loss of 9p

Total Points 0 50 100 150 200 250 300

1-year DSS

2-year DSS

3-year DSS
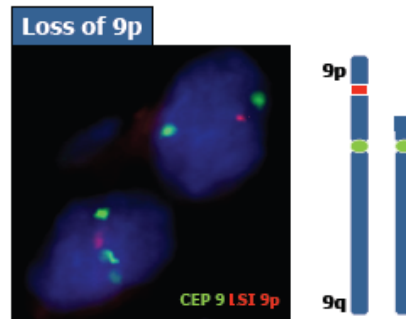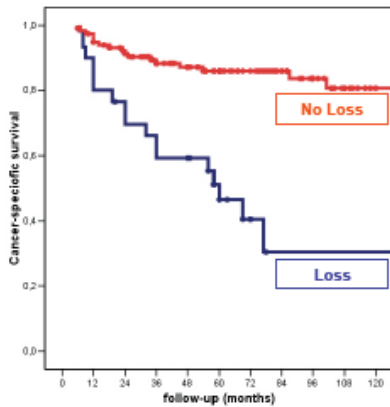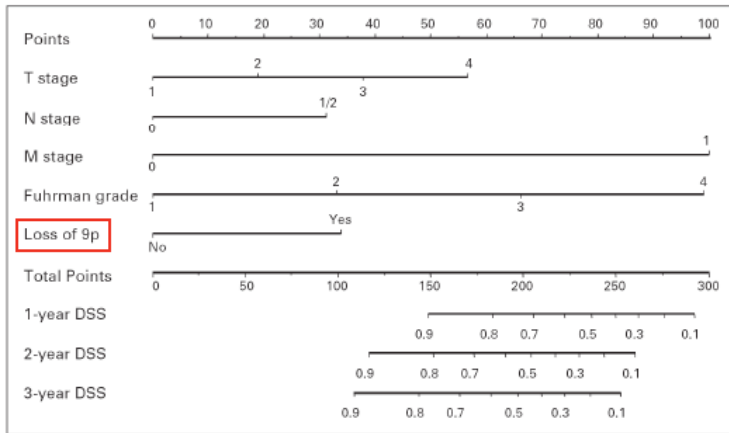
Cytogenetic profile predicts prognosis of patients with clear ce...
Klatte T, Rao PN et al. J Clin Oncol 2009;10;27

No 9p deletion

9p deletion
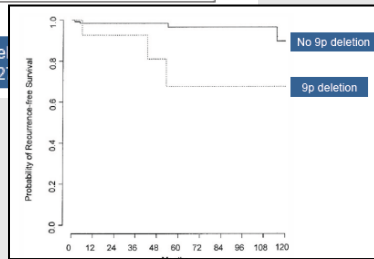
Multifocal ccRCC
46% discordant pattern>>independent origin?
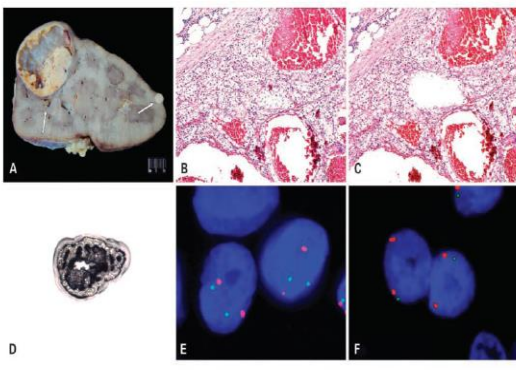
Fig. 1. Gross appearance, histology, laser capture microdissection, and FISH images of multifocal clear cell carcinoma. A, representative gross appearance of multifocal CCRCC (A) shows the tumors are confined in the kidney and located at least 1 cm apart. B, typical histology of CCRCC (B). C and D, laser capture microdissection of the same cancer tissue (C) and the tissue isolated (D). E, disomic cancer cells showed two red signals (CEP3) and three green signals (3p); F, cancer cells with chromosome arm 3p deletion showed two red signals and only one green signal.
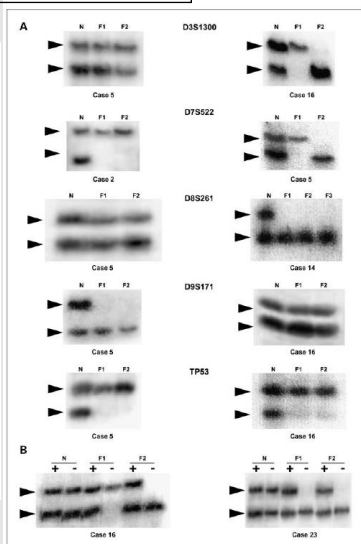
Cheng, Lopez-Beltran, Montironi.CCR, 2009

27

Multilocular cystic renal cell carcinoma is a subtype of clear cell renal cell carcinoma

Shams Halat[1], John N Eble[1], David J Grignon[1], Antonio Lopez-Beltran[3], Rodolfo Montironi[4], Puay-Hoon Tan[5], Mingsheng Wang[1], Shaobo Zhang[1], Gregory T MacLennan[6] and Liang Cheng[1,2]

A c.194C>A / p.S65X

B Microsatellite marker D3S1597 (3p)
Tumour tissue
LOH 3p

Microsatellite marker D3S1597 (3p)
Nontumour tissue

d

...photomicrograph shows the predominantly multilocular...
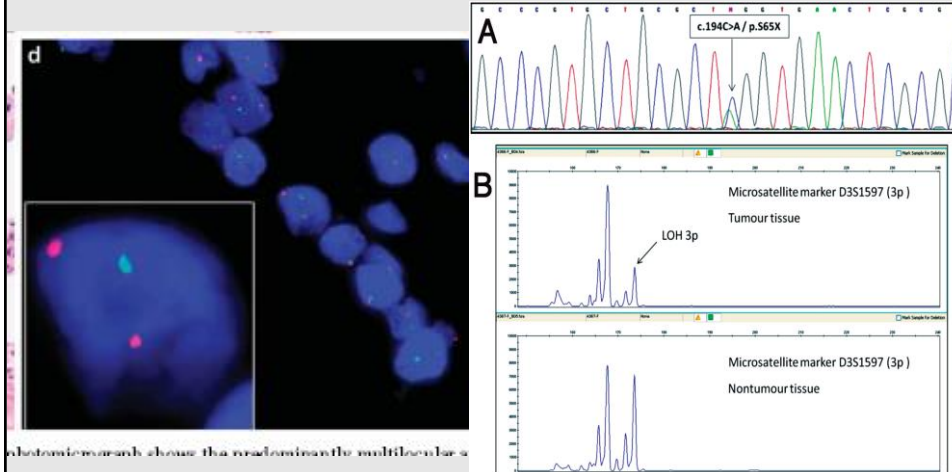
ORIGINAL ARTICLE

Unlike in clear cell renal cell carcinoma, *KRAS* is not mutated in multilocular cystic clear cell renal cell neoplasm of low potential

Maria Rosaria Raspollini[1] · Francesca Castiglione[1] · Guido Martign... · Liang Cheng[3] · Rodolfo Montironi[4] · Antonio Lopez-Beltran[5,6]

Case 1

Case 2

Case 3

Table 2 *RAS* genes pyrosequencing on selected codons investigate...

No. of case	Histological diagnosis	KRAS codon 12	KRAS codon 13
1	CCRCC	WT	p.G13D (12 %)
2	CCRCC	WT	p.G13S (16 %)
3	CCRCC	p.G12S (11 %)	WT
4	CCRCC	p.G12V (10 %)	WT
5	CCRCC	p.G12C (10 %)	WT
6	CCRCC	WT	p.G13D (40 %)
7	CCRCC	WT	p.G13D (9 %)
8	CCRCC	WT	p.G13D (9 %)
9	CCRCC	WT	p.G13D (10 %)
10	CCRCC	p.G12D (9 %)	WT
11	CCRCC	p.G12C (9 %)	WT
12	mcCCRCNLMP	WT	WT
13	mcCCRCNLMP	WT	WT
14	mcCCRCNLMP	WT	WT
15	mcCCRCNLMP	WT	WT
16	mcCCRCNLMP	WT	WT
16	mcCCRCNLMP	WT	WT
18	mcCCRCNLMP	WT	WT
19	mcCCRCNLMP	WT	WT
20	mcCCRCNLMP	WT	WT
21	mcCCRCNLMP	WT	WT
22	mcCCRCNLMP	WT	WT

Clear Cell Renal Cell Carcinoma Subtypes Identified by BAP1 and PBRM1 Expression

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Thai H. Ho,* John C. Cheville,* Eugene Frenkel,* Alexander S. Parker*
and James Brugarolas†,§

*From the Division of Medical Oncology, Departments of Health Sciences Research and Medicine and Division of Cancer Biology, Mayo Clinic Florida, Jacksonville, Florida, Department of Pathology, Kidney Cancer Program, Simmons Comprehensive Cancer Center, Division of Hematology-Oncology, Department of Internal Medicine and Department of Developmental Biology, University of Texas Southwestern Medical Center, Dallas, Texas, Department of Health Sciences Research, Mayo Clinic Rochester, Rochester, Minnesota, and Division of Medical Oncology, Mayo Clinic Arizona, Phoenix, Arizona

Abbreviations and Acronyms

BAP1 = BRCA associated protein 1
ccRCC = clear cell RCC
IHC = immunohistochemistry
PBRM1 = polybromo 1
RCC = renal cell carcinoma
RFS = relapse-free survival

Purpose: In clear cell renal cell carcinoma *BAP1* and *PBRM1* are 2 of the most commonly mutated genes (10% to 15% and 40% to 50%, respectively). We sought to determine the prognostic significance of PBRM1 and BAP1 expression in clear cell renal cell carcinoma.

Materials and Methods: We used immunohistochemistry to assess PBRM1 protein expression in 1,479 primary clear cell renal cell carcinoma tumors that were previously stained for BAP1. A centralized pathologist reviewed all cases and categorized tumors as positive or deficient for PBRM1 and BAP1. Kaplan-Meier and Cox regression models were used to evaluate association of PBRM1 and BAP1 expression with the risk of death from renal cell carcinoma and the risk of metastasis after adjustment for age and the Mayo Clinic SSIGN (stage, size, grade and necrosis) score.

Results: PBRM1 and BAP1 expression was PBRM1+ BAP1+ in 40.1% of tumors, PBRM1− BAP1+ in 48.6%, PBRM1+ BAP1− in 8.7% and PBRM1− BAP1− in 1.8%. The incidence of PBRM1 and BAP1 loss in the same tumor was significantly lower than expected (actual 1.8% vs expected 5.3%, p <0.0001). Compared to patients with PBRM1+ BAP1+ tumors those with PBRM1− BAP1+ lesions were more likely to die of renal cell carcinoma (HR 1.39, p = 0.035), followed by those with PBRM1+ BAP1− and PBRM1− BAP1− tumors (HR 3.25 and 5.2, respectively, each p <0.001). PBRM1 and BAP1 expression did not add independent prognostic information to the SSIGN score.
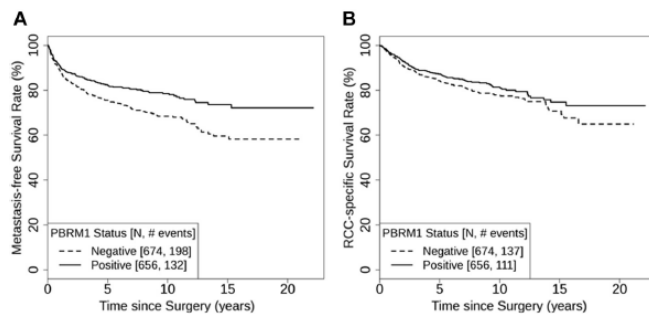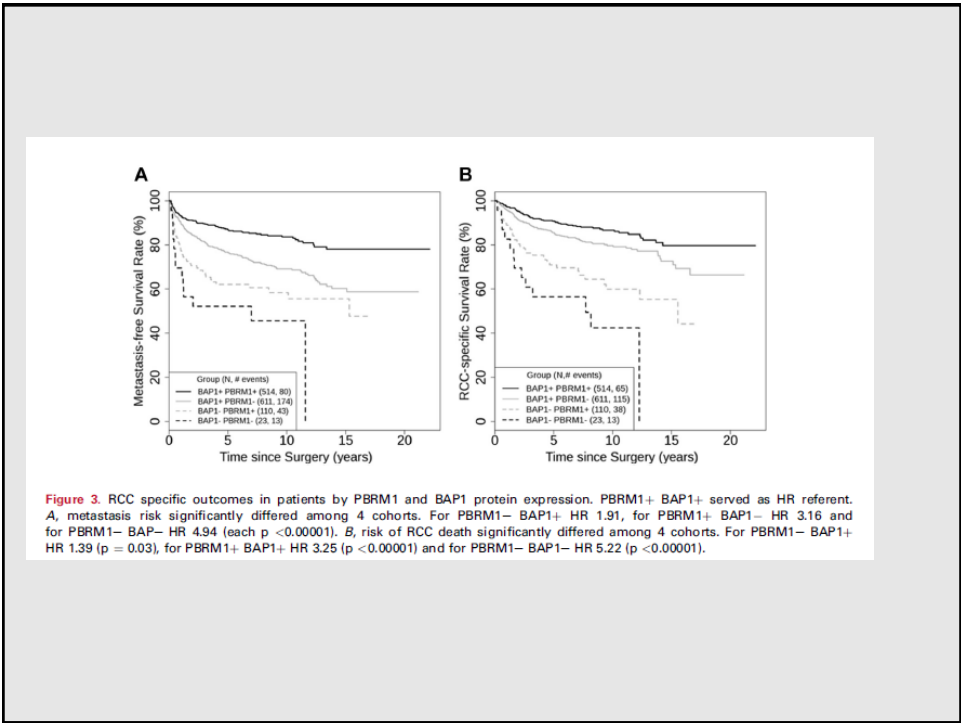
Figure 1. RCC specific outcomes in patients by PBRM1 expression. *A*, patients with PBRM1− tumors were at increased risk for metastasis vs patients with PBRM1+ tumors (HR 1.46, p = 0.001). *B*, after adjusting for age patients with PBRM1− tumors were not at increased risk for RCC death vs patients with PBRM1+ tumors (HR 1.08, p = 0.54).

Figure 3. RCC specific outcomes in patients by PBRM1 and BAP1 protein expression. PBRM1+ BAP1+ served as HR referent. A, metastasis risk significantly differed among 4 cohorts. For PBRM1− BAP1+ HR 1.91, for PBRM1+ BAP1− HR 3.16 and for PBRM1− BAP− HR 4.94 (each p <0.00001). B, risk of RCC death significantly differed among 4 cohorts. For PBRM1− BAP1+ HR 1.39 (p = 0.03), for PBRM1+ BAP1+ HR 3.25 (p <0.00001) and for PBRM1− BAP1− HR 5.22 (p <0.00001).

pRCC

Lopez-Beltran et al 2011

PRCC Immunohistochemistry

- Diffuse positivity for CK7 (more often in type 1 than in type 2)
- Racemase diffusely positive with cytoplasmic granular staining
- CD10 usually positive with luminal membranous staining

CK7

Racemase

CD10
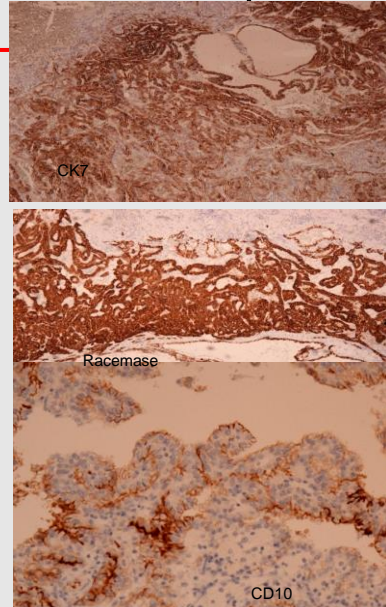
PAPILLARY RENAL CELL CARCINOMA:
Most frequent DNA sequence copy number gains

	Percent of tumors		
	Type 1 *n*=9	Type 2 *n*=16	*p value*
7p+	100	31.2	0.004
7q+	66.7	1.2	*NS*
17p+	100	7.5	0.008
17q+	100	68.8	*NS*

Jiang et al. Am J Pathol 153:1467,1998.

Papillary Carcinoma
Molecular Pathology

- Hereditary : germline mutations of the c-MET protooncogene at 7p31
- Sporadic: gains of chromosomes 7 and 17 and loss of chromosome Y in male patients
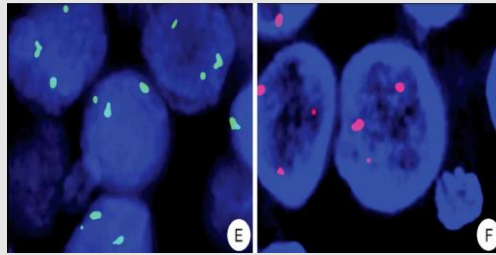
Multifocal pRCC>>patrón concordante

Jones, Lopez-Beltran, Cheng, CCR, 2005

ORIGINAL ARTICLE

Comprehensive Molecular Characterization of Papillary Renal-Cell Carcinoma

The Cancer Genome Atlas Research Network*

NEJM 374:2
Jan 2016

ABSTRACT

BACKGROUND

Papillary renal-cell carcinoma, which accounts for 15 to 20% of renal-cell carcinomas, is a heterogeneous disease that consists of various types of renal cancer, including tumors with indolent, multifocal presentation and solitary tumors with an aggressive, highly lethal phenotype. Little is known about the genetic basis of sporadic papillary renal-cell carcinoma, and no effective forms of therapy for advanced disease exist.

METHODS

We performed comprehensive molecular characterization of 161 primary papillary renal-cell carcinomas, using whole-exome sequencing, copy-number analysis, messenger RNA and microRNA sequencing, DNA-methylation analysis, and proteomic analysis.

RESULTS

Type 1 and type 2 papillary renal-cell carcinomas were shown to be different types of renal cancer characterized by specific genetic alterations, with type 2 further classified into three individual subgroups on the basis of molecular differences associated with patient survival. Type 1 tumors were associated with MET alterations, whereas type 2 tumors were characterized by CDKN2A silencing, SETD2 mutations, TFE3 fusions, and increased expression of the NRF2–antioxidant response element (ARE) pathway. A CpG island methylator phenotype (CIMP) was observed in a distinct subgroup of type 2 papillary renal-cell carcinomas that was characterized by poor survival and mutation of the gene encoding fumarate hydratase (FH).

CONCLUSIONS

Type 1 and type 2 papillary renal-cell carcinomas were shown to be clinically and biologically distinct. Alterations in the MET pathway were associated with type 1, and activation of the NRF2-ARE pathway was associated with type 2; CDKN2A loss and CIMP in type 2 conveyed a poor prognosis. Furthermore, type 2 papillary renal-cell carcinoma consisted of at least three subtypes based on molecular and phenotypic features. (Funded by the National Institutes of Health.)

Figure 1 (facing page). Somatic Alterations in Papillary Renal-Cell Carcinoma and Molecular Differences between Type 1 and Type 2 Cancers.

Unsupervised clustering of DNA copy profiles of 161 papillary renal-cell carcinomas (PRCCs) (Panel A) revealed three molecular subtypes, one of which was highly enriched for type 1 tumors and the other two for type 2 tumors. SCNA denotes somatic copy-number alterations. Significantly mutated genes (SMGs) in PRCC (Panel B) were determined by considering all genes (q<0.1 [range, 0.0 to 1.0]) or focusing on the set of 260 genes previously implicated in cancer by large-scale, pan-cancer exome analyses[15] (q<0.1). P values were calculated with the MutSigCV algorithm, version 2.0. A pathway-centric view of gene mutations in PRCC (Panel C) shows key pathways and genes implicated in cancer, either in the current study or elsewhere.[15] The tumors were classified according to histologic type (from left to right) and according to gene or pathway altered (from top to bottom). Pathways and genes represented include MET, the Hippo pathway (NF2, SAV1, and WWC1), the NRF2 pathway (NFE2L2, KEAP1, CUL3, SIRT1, and FH), chromatin modification (CREBBP, DOTL1, EHMT1/2, EP300, EZH1/2, KAT2A/B, KDM1A/B, KDM4A/B, KDM5A/B/C, KDM6A/B, MLL1/2/3/4/5, NSD1, SETD2, SMYD4, and SRCAP), the SWI/SNF complex (ACTB, ACTL6A/B, ARID1A/B, ARID2, BCL6A/B/C, BCL11A/B, BRD7/9, DPF1/2/3, PHF10, PBRM1, SMARCA2/4, SMARCB1, SMARCC1/2, SMARCD1/2/3, and SMARCE1), the mammalian target of rapamycin (mTOR) pathway (MTOR, PIK3CA, PTEN, STK11, TSC1, and TSC2), and the p53 pathway (ATM, CDKN1A, CDKN2A, FBXW7, RB1, and TP53). Fusion gene analysis (Panel D) identified TFE3 or TFEB fusions in eight PRCC tumors, including two novel gene-fusion partners for TFE3 (DVL2 and RBM10) and two novel gene-fusion partners for TFEB (COL21A1 and CADM2). Schematic versions of these fusions show the exons and functional domains that are present in the different gene fusions and the position of potential microRNA binding sites in TFEB. The retained exons of TFE3 or TFEB are colored in shades of blue. Thin regions represent noncoding sequence, thick regions represent the translated reading frame, and white strips indicate that the region is no longer to scale. AD denotes strong transcription activation domain, bHLH basic helix-loop-helix domain, DIX dishevelled and axin domain, LZ leucine zipper domain, MAD2L2 mitotic arrest deficient–like 2 interaction domain, and RRM RNA-recognition motif.
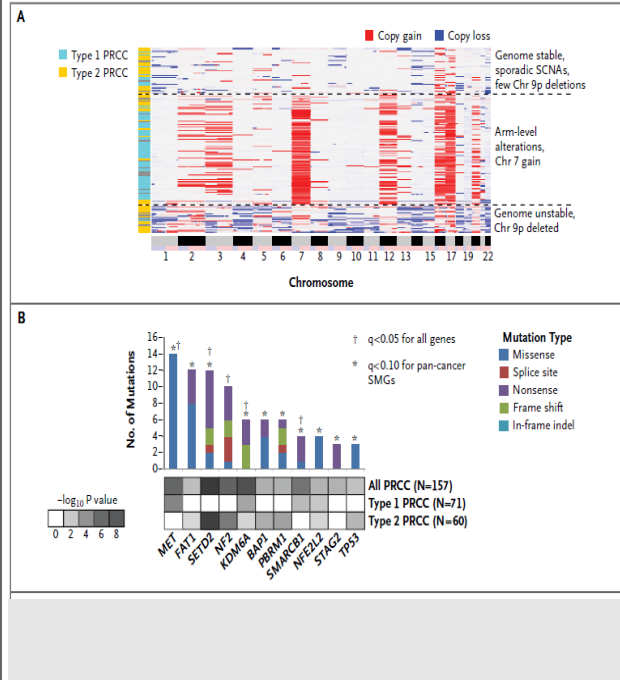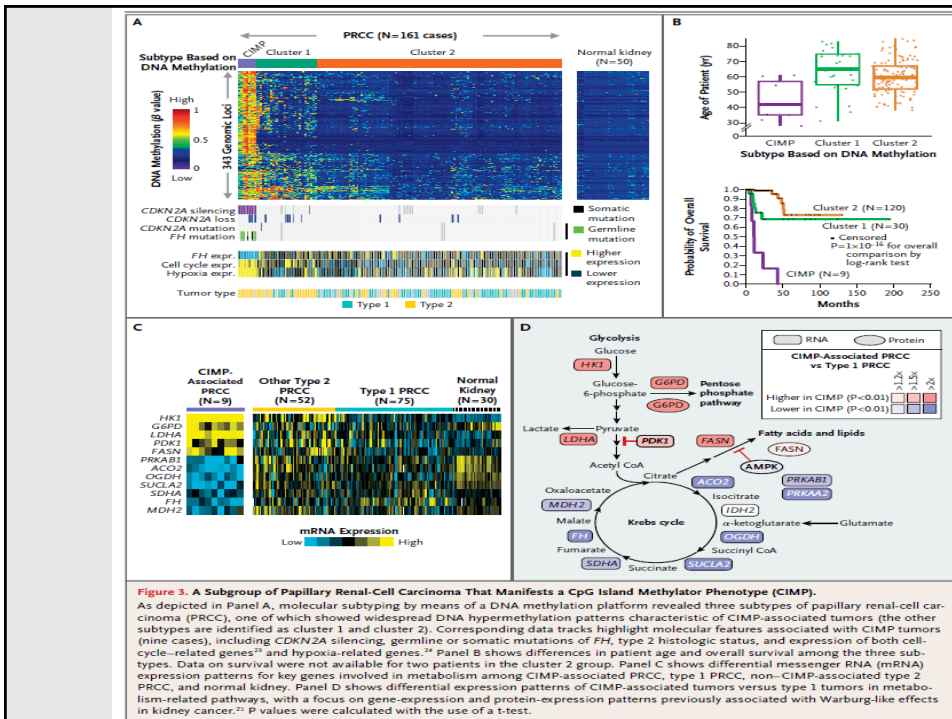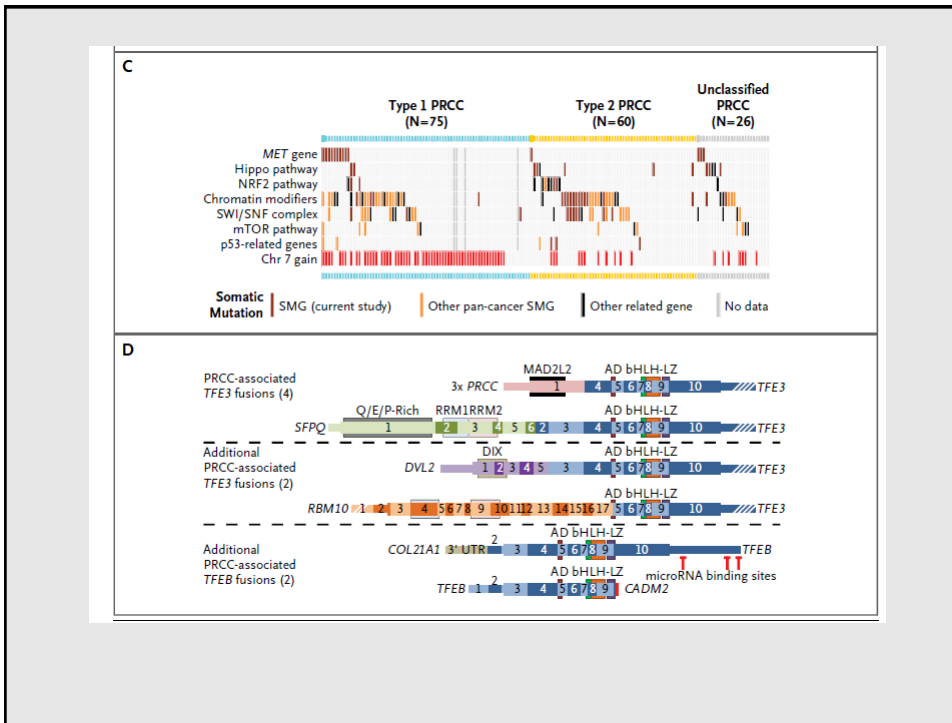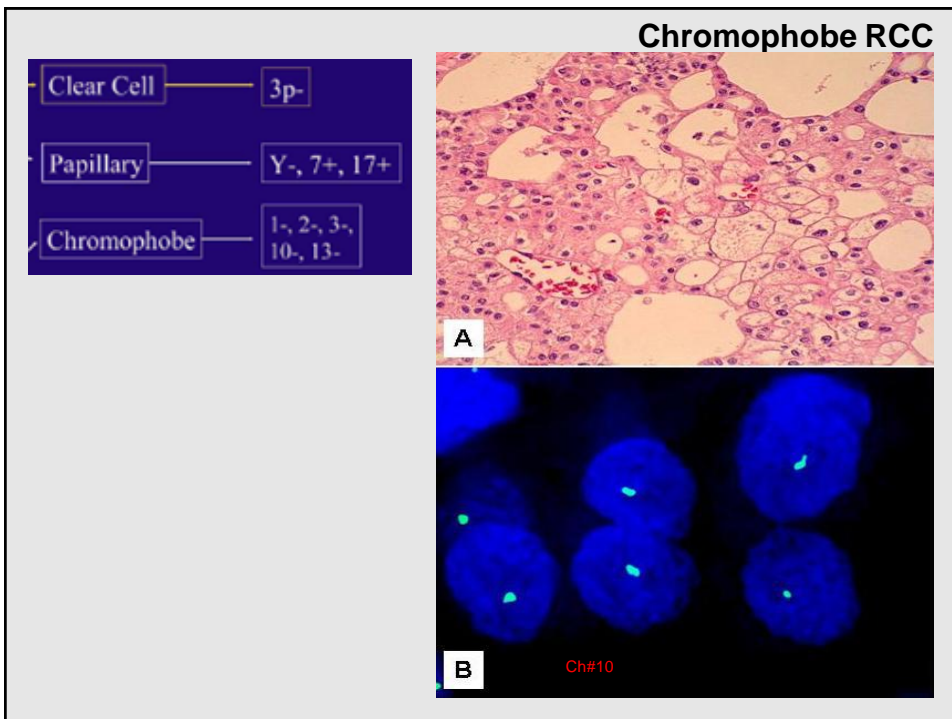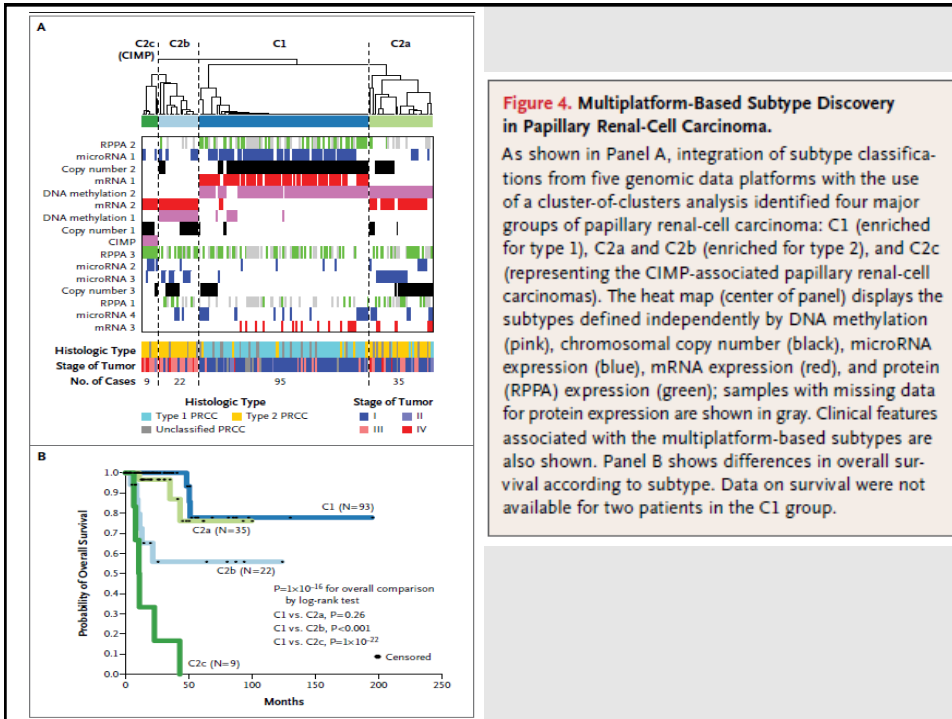
Figure 3. A Subgroup of Papillary Renal-Cell Carcinoma That Manifests a CpG Island Methylator Phenotype (CIMP).

As depicted in Panel A, molecular subtyping by means of a DNA methylation platform revealed three subtypes of papillary renal-cell carcinoma (PRCC), one of which showed widespread DNA hypermethylation patterns characteristic of CIMP-associated tumors (the other subtypes are identified as cluster 1 and cluster 2). Corresponding data tracks highlight molecular features associated with CIMP tumors (nine cases), including CDKN2A silencing, germline or somatic mutations of FH, type 2 histologic status, and expression of both cell-cycle–related genes[23] and hypoxia-related genes.[24] Panel B shows differences in patient age and overall survival among the three subtypes. Data on survival were not available for two patients in the cluster 2 group. Panel C shows differential messenger RNA (mRNA) expression patterns for key genes involved in metabolism among CIMP-associated PRCC, type 1 PRCC, non–CIMP-associated type 2 PRCC, and normal kidney. Panel D shows differential expression patterns of CIMP-associated tumors versus type 1 tumors in metabolism-related pathways, with a focus on gene-expression and protein-expression patterns previously associated with Warburg-like effects in kidney cancer.[25] P values were calculated with the use of a t-test.

Figure 4. Multiplatform-Based Subtype Discovery in Papillary Renal-Cell Carcinoma.

As shown in Panel A, integration of subtype classifications from five genomic data platforms with the use of a cluster-of-clusters analysis identified four major groups of papillary renal-cell carcinoma: C1 (enriched for type 1), C2a and C2b (enriched for type 2), and C2c (representing the CIMP-associated papillary renal-cell carcinomas). The heat map (center of panel) displays the subtypes defined independently by DNA methylation (pink), chromosomal copy number (black), microRNA expression (blue), mRNA expression (red), and protein (RPPA) expression (green); samples with missing data for protein expression are shown in gray. Clinical features associated with the multiplatform-based subtypes are also shown. Panel B shows differences in overall survival according to subtype. Data on survival were not available for two patients in the C1 group.

Chromophobe RCC

Chromophobe Carcinoma Immunohistochemistry

- Diffuse expression of CK7 with membranous accentuation
- CD117 diffusely positive
- CD10 negative
- Other positive markers: Claudin-7, Ksp Cadherin, CD82

CK7

CD117
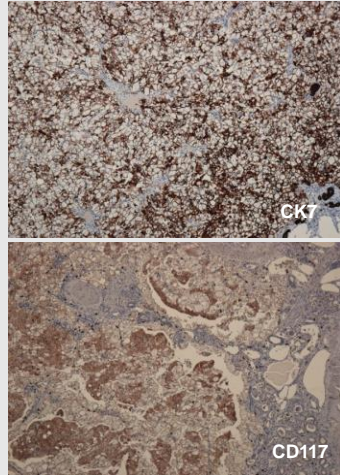
Sarcomatoid Differentiation in Renal cell Carcinoma

- 8% in clear cell
- 3% in papillary
- 9% in chromophobe
- 29% in collecting duct
- 11% in unclassified
- 95% of cases nuclear grade 3 or 4

Carcinoma
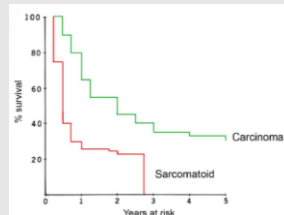
Sarcomatoid

Peralta-Venturina et al., 2001

Genomic characterization of sarcomatoid transformation in clear cell renal cell carcinoma

Mark Bi[a,b], Siming Zhao[a,b], Jonathan W. Said[c], Maria J. Merino[d], Adebowale J. Adeniran[e], Zuoquan Xie[f], Cayce B. Nawaf[f], Jaehyuk Choi[g], Arie S. Belldegrun[h], Allan J. Pantuck[h], Harriet M. Kluger[i], Kaya Bilgüvar[a], Richard P. Lifton[a,b,1], and Brian Shuch[c,1]

The presence of sarcomatoid features in clear cell renal cell carcinoma (ccRCC) confers a poor prognosis and is of unknown pathogenesis. We performed exome sequencing of matched normal-carcinomatous-sarcomatoid specimens from 21 subjects. Two tumors had hypermutation consistent with mismatch repair deficiency. In the remainder, sarcomatoid and carcinomatous elements shared 42% of somatic single-nucleotide variants (SSNVs). Sarcomatoid elements had a higher overall SSNV burden (mean 90 vs. 63 SSNVs, $P = 4.0 \times 10^{-4}$), increased frequency of nonsynonymous SSNVs in Pan-Cancer genes (mean 1.4 vs. 0.26, $P = 0.002$), and increased frequency of loss of heterozygosity (LOH) across the genome (median 913 vs. 460 Mb in LOH, $P < 0.05$), with significant recurrent LOH on chromosomes 1p, 9, 10, 14, 17p, 18, and 22. The most frequent SSNVs shared by carcinomatous and sarcomatoid elements were in known ccRCC genes including von Hippel–Lindau tumor suppressor (VHL), polybromo 1 (PBRM1), SET domain containing 2 (SETD2), phosphatase and tensin homolog (PTEN). Most interestingly, sarcomatoid elements acquired biallelic tumor protein p53 (TP53) mutations in 32% of tumors ($P = 5.47 \times 10^{-17}$); TP53 mutations were absent in carcinomatous elements in nonhypermutated tumors and rare in previously studied ccRCCs. Mutations in known cancer drivers AT-rich interaction domain 1A (ARID1A) and BRCA1 associated protein 1 (BAP1) were significantly mutated in sarcomatoid elements and were mutually exclusive with TP53 and each other. These findings provide evidence that sarcomatoid elements arise from dedifferentiation of carcinomatous ccRCCs and implicate specific genes in this process. These findings have implications for the treatment of patients with these poor-prognosis cancers.
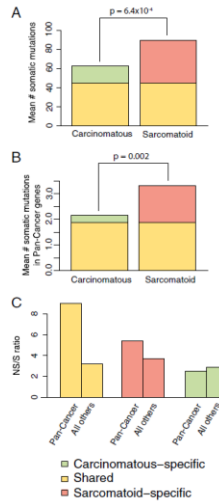
Molecular Pathology of clear cell RCC
Therapeutic implications

Gene Name	Sample Number	Positive Samples	Percent Mutated
VHL	2064	956	46%
CDKN2A	251	24	9%
PTEN	201	10	4%
PML*	288	3	1%
AKAP4	96	3	3%

ccRCC

Mutations observed in ccRCC. Adapted from Wellcome Trust Sanger Institute (http://www.sanger.ac.uk/). (*) Number of positive samples should be 1. Reproduced with permission.

Pathways of VHL-associated carcinogenesis.

38
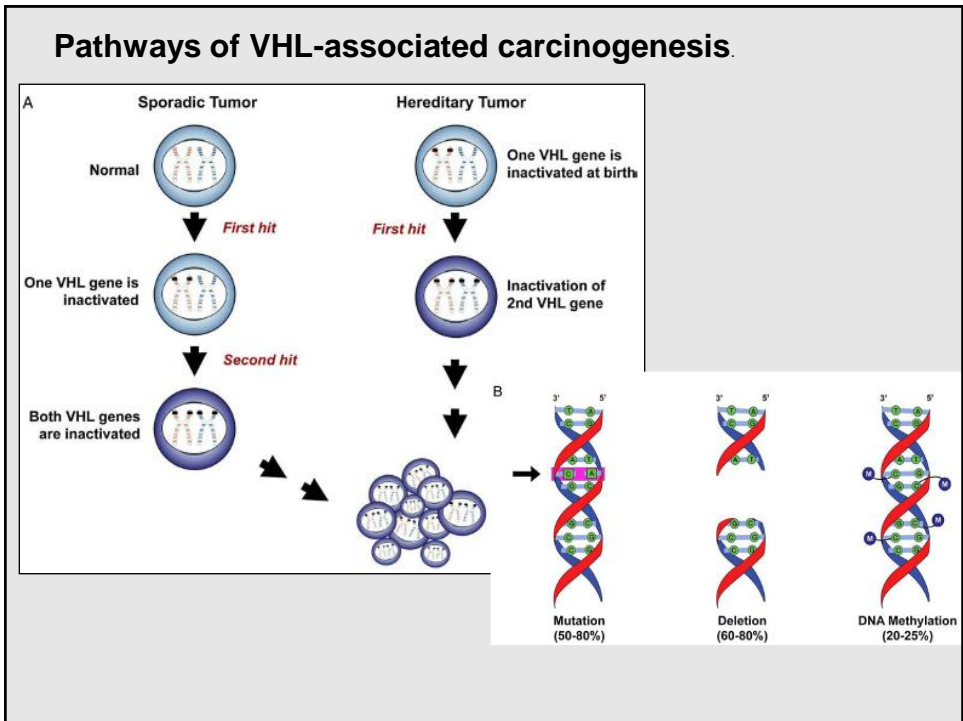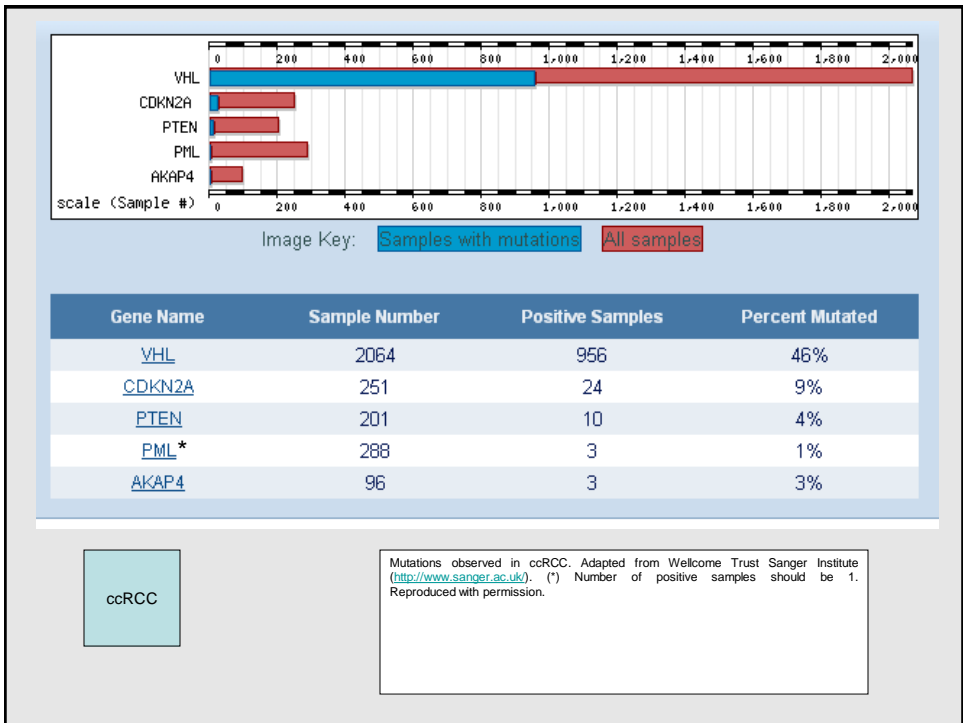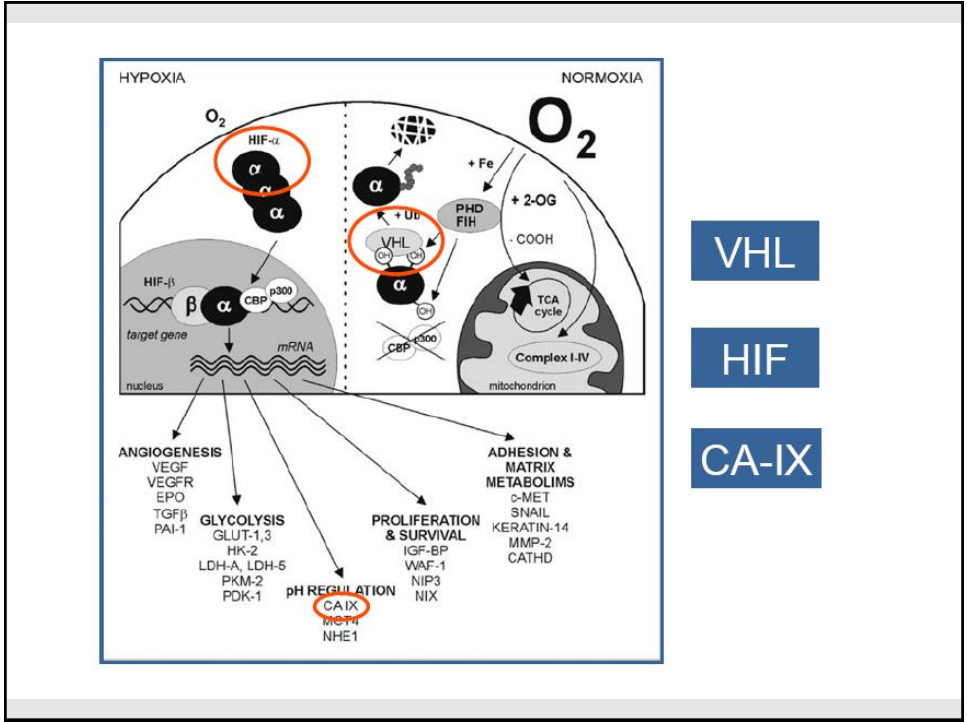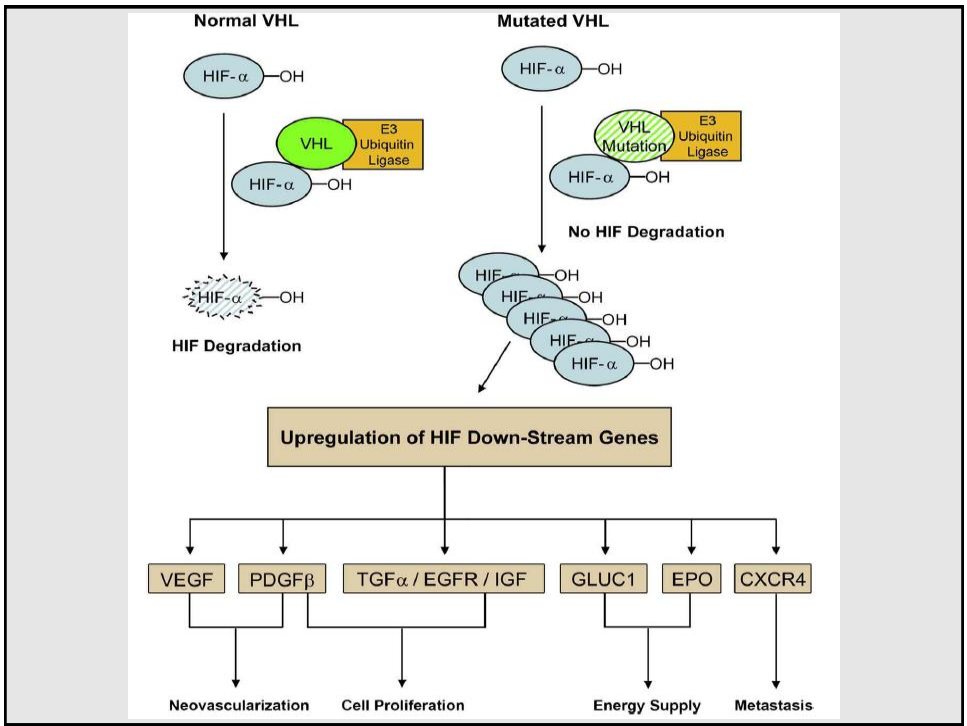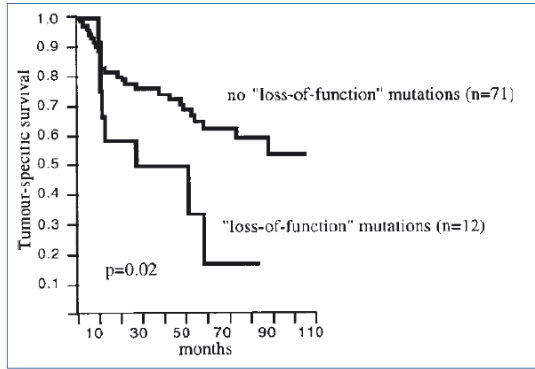
VHL mutations and their correlation with tumour cell proliferation, microvessel density, and patient prognosis in clear cell renal cell carcinoma.
Peter Schraml et al. J Pathol 2002;196:186
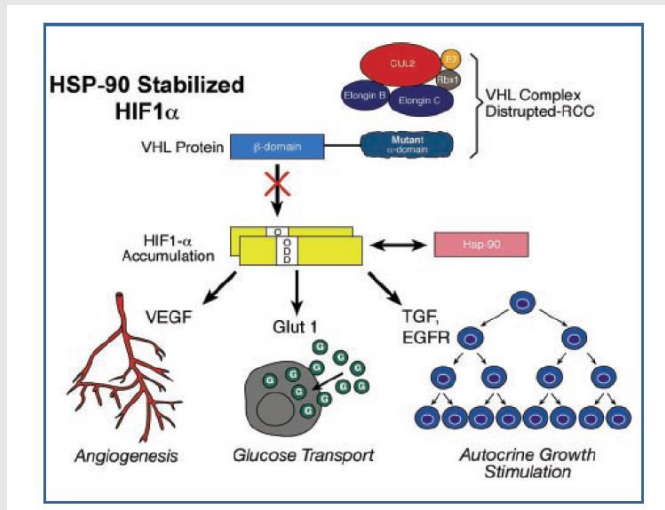
Hypoxia Inducible Factor (HIF)
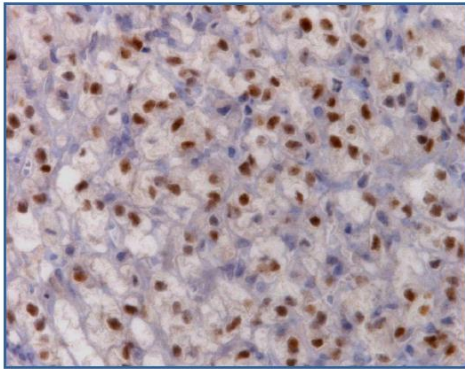
Nuclear expression of hypoxia-inducible factor-1alpha in clear cell renal cell carcinoma is involved in tumor progression

Di Cristofano et Al. Am J Surg Pathol. 2007:1875-81
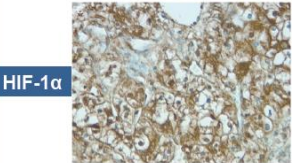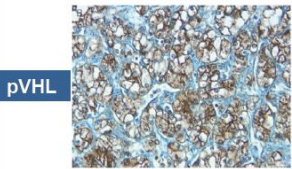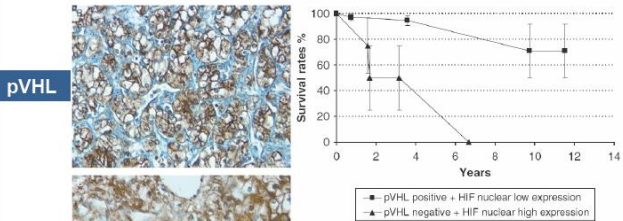
Hypoxia-inducible factor 1alpha expression in renal cell carcinoma analyzed by tissue microarray.

Lidgren et al. Eur Urol. 2006;50:1272.

HIF-1α-LOW   HIF-1α-HIGH
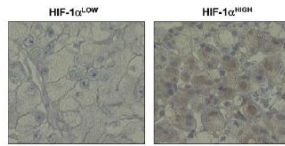
"patients with high HIF-1a levels tended to have a better prognosis."

Hypoxia-inducible factor 1 alpha in clear cell renal cell carcinoma.

Klatte et al. Clin Cancer Res. 2007;13:7388.

Normal   Low Grade   High Grade
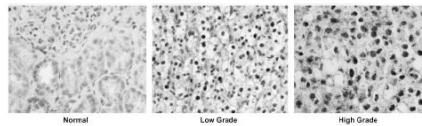
"patients with high HIF-1a expression had significantly worse survival than patients with low expression"

ccRCC Molecular Pathways

- Inactivation of VHL<<<Two key pathways essential to the pathophysiology of the ccRCC:
- (1) **the hypoxia-inducible pathway** associated with frequent mutations of the von Hippel–Lindau (VHL) tumor suppressor gene
- (2) **the mTOR (mammalian target of rapamycin) signaling pathway**.
- Inhibitors targeting various aspects of these pathways support the onset of a new therapeutic era for patients with metastatic ccRCC.

Eichelberg et al. Eur Urol 2009;851-630

Pathways of VHL-associated carcinogenesis.

g. (1). VEGF and mTOR pathways and the role for targeted therapy in renal cell carcinoma.
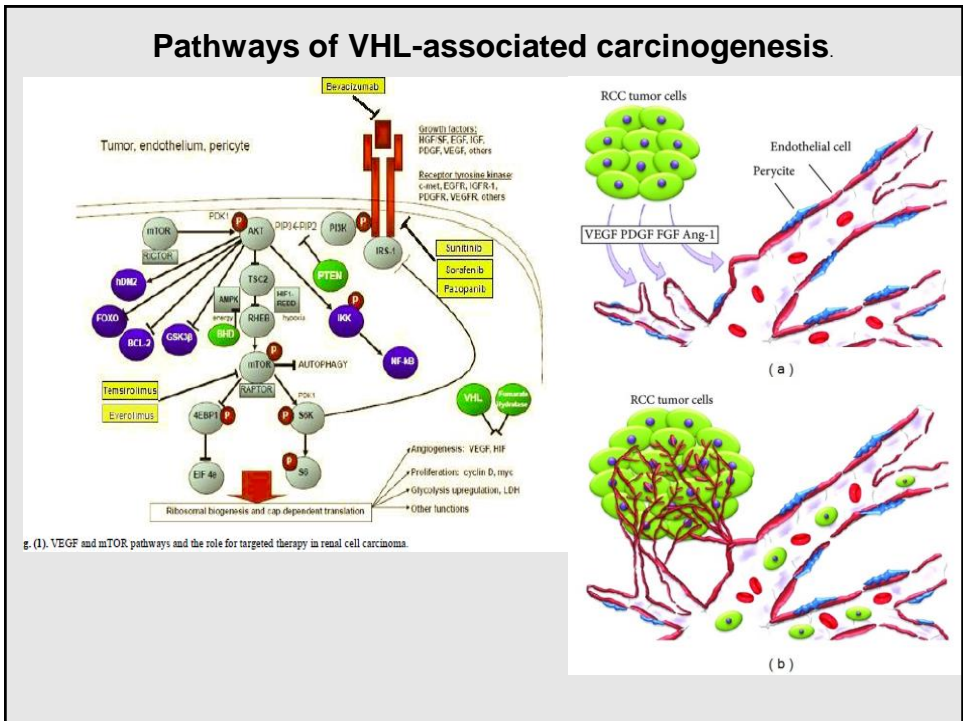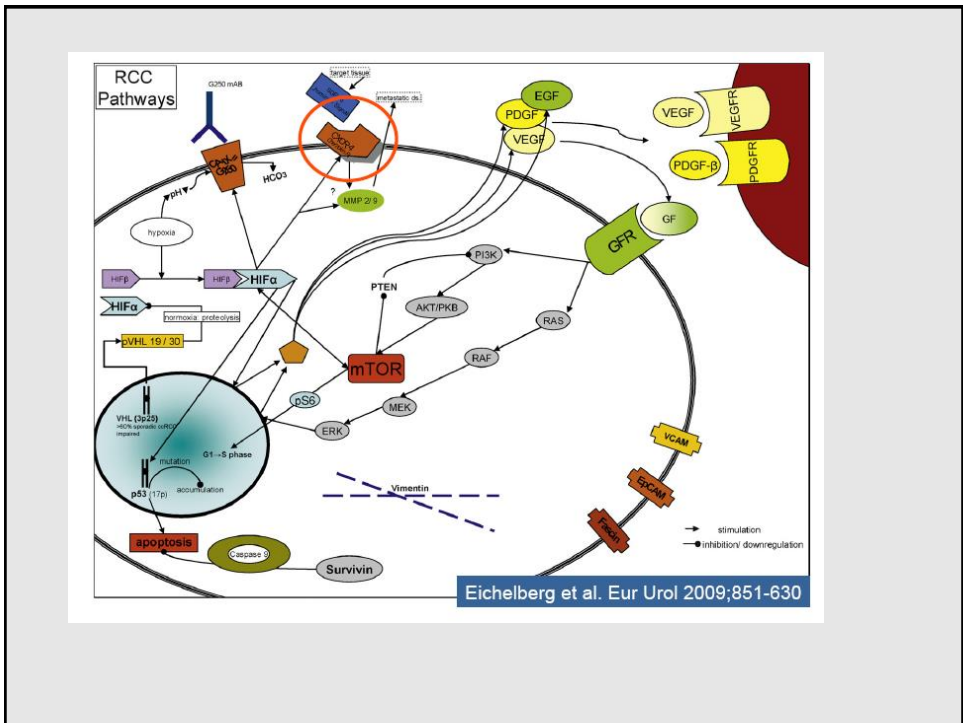
Terapêutica Sistémica - mTORi
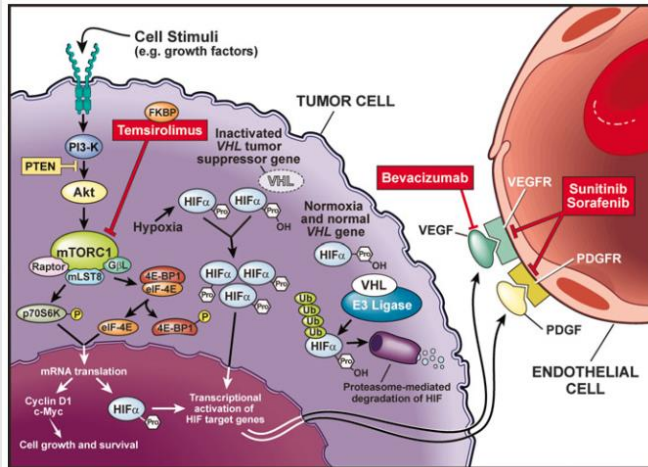
- **Tensirolimus**
- **Everolimus**

Nargund VH et al. "Urological Oncology, 2nd Edition"

Terapêutica Sistémica - Anti-VEGF
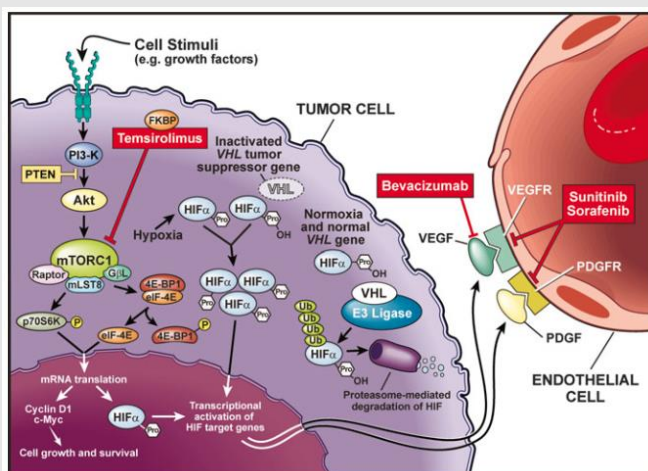
- Ac monoclonais
  - **Bevacizumab**

- Pequenas moléculas
  - **Sunitinib**
  - **Pazopanib**
  - **Sorafenib**
  - **Axitinib**
  - **Cabozantinib**

Nargund VH et al. "Urological Oncology, 2nd Edition"

Molecular Pathology of RCC

Table 3   Molecular targets of current targeted therapies in RCC and other urologic tumors

Target	Sunitinib	Sorafenib	Bevacizumab	Temsirolimus
VEGF	No inhibition	No inhibition	Inhibition	No inhibition
VEGFR1 (Flt-1)	Inhibits target	No inhibition	No inhibition	No inhibition
VEGFR2 (Flk-1/KDR)	No inhibition	Inhibits target	No inhibition	No inhibition
VEGFR3 (Flt-4)	Inhibits target	Inhibits target	No inhibition	No inhibition
PDGFR-α	No inhibition	No inhibition	No inhibition	No inhibition
PDGFR-β	Inhibits target	Inhibits target	No inhibition	No inhibition
c-kit	Inhibits target	Inhibits target	No inhibition	No inhibition
FLT-3	Inhibits target	Inhibits target	No inhibition	No inhibition
SCFR	Inhibits target	No inhibition	No inhibition	No inhibition
RET	Inhibits target	No inhibition	No inhibition	No inhibition
FAK	No inhibition	No inhibition	No inhibition	No inhibition
b-FGF	No inhibition	No inhibition	No inhibition	No inhibition
B-raf kinase	No inhibition	Inhibits target	No inhibition	No inhibition
C-raf kinase	No inhibition	Inhibits target	No inhibition	No inhibition
mTOR	No inhibition	No inhibition	No inhibition	Inhibits target

López-Beltran et al 2008

TABLE II. *Potential molecular markers for renal cell carcinoma*

Hypoxia Inducible	Proliferation	Cell Cycle Regulation	Cell Adhesion	Miscellaneous
• CAIX	• Ki-67	• p53	• EpCAM	• Gelsolin
• CAXII	• PCNA	• bcl-2	• EMA	• Vimentin
• CXCR-4	• Ag-NORs	• PTEN	• E-cadherin	• CA-125
• HIF-1α		• Cyclin A	• α-Catenin	• CD44
• VEGF		• Akt	• Cadherin-6	• Androgen receptors
• IGF-I		• S6 kinase		• Caveolin-1
		• p27		• VEGF-R
				• Na$^+$/K$^+$ ATPase subunits
				• DNA ploidy

ATPase = adenosine triphosphatase; CAIX = carbonic anhydrase IX; CAXII = carbonic anhydrase XII; CXCR-4 = CXC chemokine receptor–4; EMA = ; EpCAM = epithelial cell adhesion molecule; HIF-1α = hypoxia-inducible factor–1α; IGF-1 = insulin-like growth factor–1; PTEN = phosphatase and tensin homolog deleted on chromosome 10; VEGF = vascular endothelial growth factor; VEGF-R = VEGF receptor.
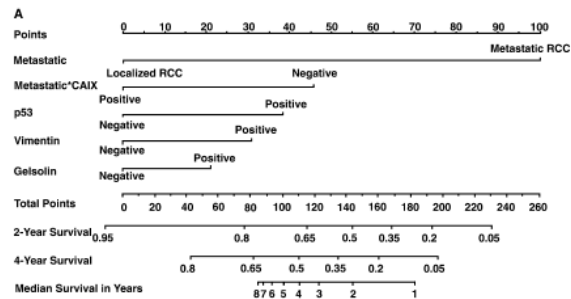
Gene Expression Profiling

- Gene expression profiling study, 31 adult renal tumors (including 13 clear cell renal cell carcinomas, 5 papillary renal cell carcinomas, 4 chromophobe renal cell carcinomas, 3 oncocytomas, and 6 angiomyolipomas) were analyzed.
- The authors found that clear cell renal cell carcinomas, chromophobe renal cell carcinoma, and papillary renal cell carcinomas expressed different panel of genes, which correlated with cellular origin of the tumors. Shuetz et al JMD 2008

Gene Expression Profiling

- Large series of 65 cases >>gene expression profiling can identify tumor subtypes with100% accuracy
  - a unique metastatic signature can be identified for renal cell carcinomas. Jones et al CCR2005
- In another study, 112 renal cell carcinomas and normal kidney samples were analyzed for gene expression profiling.
- The gene expression patterns showed that the molecular changes corresponded well to the histopathologic tumor types, and a set of 80 genes was sufficient to classify tumors with a very low error rate.
- Distinct gene expression signatures were associated with chromosomal abnormalities of tumorcells, metastasis formation, and patient survival Sultman et al CCR 2005

Gene Expression Profiling

AP Clinical Service Integrated View

AP Clinical Service Integrated View

ABL1	BARD1	CD79A	CSF1R	EZH2	FGFR2	HRAS	KEAP1	MLL2	NRAS	PRKDC	SMARCB1	TSC2
AKT1	BCL2	CD79B	CTCF	FAM123B (WTX)	FGFR3	IDH1	KIT	MPL	NTRK1	PTCH1	SMO	TSHR
AKT2	BCL2L2	CDC73	CTNNA1	FAM46C	FGFR4	IDH2	KLHL6	MRE11A	NTRK2	PTEN	SOCS1	VHL
AKT3	BCL6	CDH1	CTNNB1	FANCA	FLT1	IGF1R	KRAS	MSH2	NTRK3	PTPN11	SOX10	WISP3
ALK	BCOR	CDK12	DAXX	FANCC	FLT3	IKBKE	LRP1B	MSH6	NUP93	RAD50	SOX2	WT1
APC	BCORL1	CDK4	DDR2	FANCD2	FLT4	IKZF1	MAP2K1	MTOR	PAK3	RAD51	SPEN	XPO1
AR	BLM	CDK6	DNMT3A	FANCE	FOXL2	IL7R	MAP2K2	MUTYH	PALB2	RAF1	SPOP	ZNF217
ARAF	BRAF	CDK8	DOT1L	FANCF	GATA1	INHBA	MAP2K4	MYC	PAX5	RARA	SRC	ZNF703
ARFRP1	BRCA1	CDKN1B	EGFR	FANCG	GATA2	IRF4	MAP3K1	MYCL1	PBRM1	RB1	STAG2	
ARID1A	BRCA2	CDKN2A	EMSY (C11orf30)	FANCL	GATA3	IRS2	MCL1	MYCN	PDGFRA	RET	STAT4	
ARID2	BRIP1	CDKN2B	EP300	FBXW7	GID4 (C17orf39)	JAK1	MDM2	MYD88	PDGFRB	RICTOR	STK11	
ASXL1	BTK	CDKN2C	EPHA3	FGF10	GNA11	JAK2	MDM4	NF1	PDK1	RNF43	SUFU	
ATM	CARD11	CEBPA	EPHA5	FGF14	GNA13	JAK3	MED12	NF2	PIK3CA	RPTOR	TET2	
ATR	CBFB	CHEK1	EPHB1	FGF19	GNAQ	JUN	MEF2B	NFE2L2	PIK3CG	RUNX1	TGFBR2	
ATRX	CBL	CHEK2	ERBB2	FGF23	GNAS	KAT6A (MYST3)	MEN1	NFKBIA	PIK3R1	SETD2	TNFAIP3	
AURKA	CCND1	CIC	ERBB3	FGF3	GPR124	KDM5A	MET	NKX2-1	PIK3R2	SF3B1	TNFRSF14	
AURKB	CCND2	CREBBP	ERBB4	FGF4	GRIN2A	KDM5C	MITF	NOTCH1	PPP2R1A	SMAD2	TOP1	
AXL	CCND3	CRKL	ERG	FGF6	GSK3B	KDM6A	MLH1	NOTCH2	PRDM1	SMAD4	TP53	
BAP1	CCNE1	CRLF2	ESR1	FGFR1	HGF	KDR	MLL	NPM1	PRKAR1A	SMARCA4	TSC1	

Select Rearrangements

ALK	BCL2	BCR	BRAF	EGFR	ETV1	ETV4	ETV5	ETV6	EWSR1	MLL	MYC	NTRK1
PDGFRA	RAF1	RARA	RET	ROS1	TMPRSS2							

TABLE 1: Novel targeted agents currently under evaluation for mRCC.

| Agent	Description	Trial ID number	Phase	Design
Brivanib	Dual VEGFR2 and FGFR-1	NCT01253668	II	RCC patients after prior treatment with TKI or bevacizumab
Crizotinib	Alk and c-MET TKI	NCT01524926	II	Patients with solid tumors
BIBF 120	VEGFR 1–3 PDGFR and FGFR TKI	NCT01024920	II	versus sunitinib in untreated mRCC patients
VEGF-Trap	Soluble decoy receptor; derivative of VEGFR1	NCT00357760	II	ccRCC patients after at least 1 prior treatment with TKI
Ridaforolimus	MTORC1 selective inhibitor	NCT01169532	I	In combination with *vorinostat* in patients with solid tumors
		NCT01295632	I	In combination with *MK2206* or γ-secretase inhibitor *MK-0752* in patients with advanced solid tumors
MK-2206	AKT inhibitor	NCT01239342	II	Versus everolimus in refractory RCC patients
NVP-BEZ235	Dual PI3K/mTOR inhibitor	NCT01482156	I	In combination with everolimus in patients with advanced solid tumors
GDC-0980	Dual PI3K/mTOR inhibitor	NCT01442090	II	In comparison with *everolimus* in mRCC patients progressed on VEGF-targeted therapy
AMG-386	Ang-1/2 inhibitor	NCT01548482	II	In combination with *temsirolimus* in patients with advanced solid tumors
MDX-1203	Anti-CD70 Ab-drug conjugate	NCT00944905	I	Pretreated ccRCC or B-cell non-Hodgkin's lymphoma
MDX-1411	Anti-CD70 Ab-drug conjugate	NCT00656734	I	ccRCC pts treated with up to 6 prior systemic therapies
SGN-75	Anti-CD70 Ab-drug conjugate	NCT01015911	I	Pretreated ccRCC or B-cell non-Hodgkin's lymphoma
Girentuximab	Chimeric mAb *cG250*	NCT00087022	III	Adjuvant cG250 versus placebo in pts with ccRCC and high risk of recurrence
cG250-Lu177	Lutetium-177 labeled cG250	NCT00142415	II	pts with advanced and progressive ccRCC
90Y-cG250	Yttrium-90 labeled cG250	NCT00199875	I	pts with advanced and progressive ccRCC
Panitumumab	Anti-EGFR mAb	NCT00425035	II	mRCC pts naïve or after cytokine treatment
Vorinostat	HDAC inhibitor	NCT00278395	II	mRCC pts naïve or after cytokine treatment
RO4929097	γ-secretase/Notch inhibitor	NCT01141569	II	ccRCC pts after anti-VEGF and/or mTOR inhibitor and/or immunotherapy failure
AS1411	26-mer DNA aptamer	NCT00740441	II	ccRCC pts after at least 1 prior treatment with TKI

Other problems in the Horizon

Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing
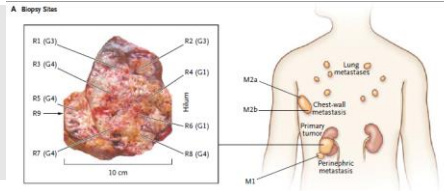

CONCLUSIONS

Intratumor heterogeneity can lead to underestimation of the tumor genomics landscape portrayed from single tumor-biopsy samples and may present major challenges to personalized-medicine and biomarker development. Intratumor heterogeneity, associated with heterogeneous protein function, may foster tumor adaptation and therapeutic failure through Darwinian selection. (Funded by the Medical Research Council and others.)

Doença

Heterogeneity

Hospedeiro   Tratamento
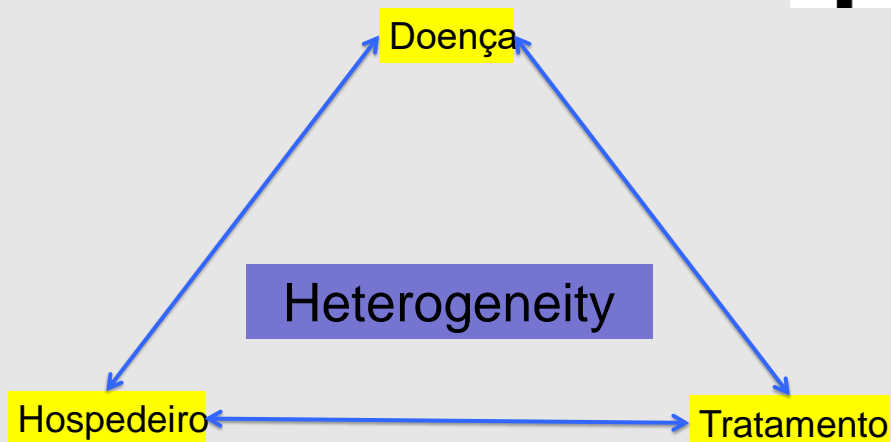
Heterogeneity – Cellular level

Nature Reviews | Cancer
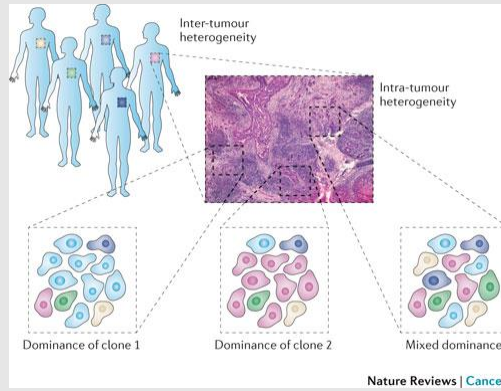
Heterogeneidade - Celular

The NEW ENGLAND JOURNAL of MEDICINE

Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing

Heterogeneity - Cellular

The NEW ENGLAND JOURNAL of MEDICINE

Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing

C Phylogenetic Relationships of Tumor Regions
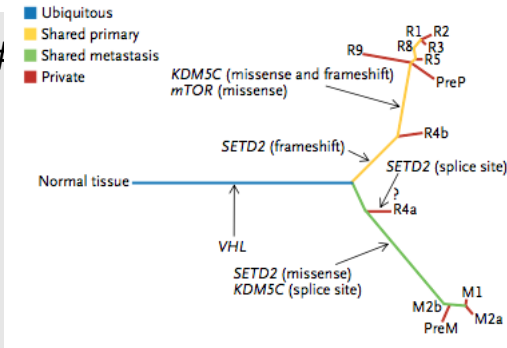
Heterogeneity - Histológic

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Platinum Priority – Collaborative Review – Kidney Cancer
Editorial by Axel Bex on pp. 98–99 of this issue

Understanding Pathologic Variants of Renal Cell Carcinoma: Distilling Therapeutic Opportunities from Biologic Complexity

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Heterogeneidade - Histológica

Table 2 – Common histologic renal cell carcinoma subtypes and their appearance and associated molecular alterations

Tumor type	Subtype*	Gross appearance	Microscopic appearance		Known somatic alterations	Cytogenetic alterations
Clear cell	–	Yellow, well circumscribed, and can possess distinct areas of hemorrhage and necrosis	Abundant clear cytoplasm due to deposition of lipid and glycogen		*VHL, PBRM1, SETD2, BAP1, JARID1A, mTOR, PI3K*	3p (90%), 14q, 8p, and 9p and gains at 5q and 12q
Papillary	1	Mixed cystic/solid consistency. Papillary RCC lesions are often reddish-brown and frequently have a well-demarcated pseudocapsule	Papillary or tubulopapillary architecture. Calcifications, necrosis, and foamy macrophage infiltration.	Type 1: thin, basophilic papillae with clear cytoplasm	*MET NRF2, CUL3*	Gains of 7, 8q, 12q, 16p, 17, 20, and loss of 9p. Papillary type 2 with gains of 8q, loss of 1p and 9p.
	2			Type 2: heterogenous, thicker papillae and eosinophilic cytoplasm.		
Chromophobe	Classic Eosinophilic	Large, well-circumscribed, tan-brown tumor with occasional central scar	Distinct cell borders and a voluminous cytoplasm, nuclear morphology with perinuclear halos, binucleation	Classic: pale cytoplasm Eosinophilic: large tumor cells with fine eosinophilic granules	*TP53*	Loss of chromosomes 1, 2, 6, 10, 13, and 17
Oncocytoma	–	Mahogany color, well circumscribed, occasional central scar, and rarely with necrosis	Polygonal cell with abundant eosinophilic cytoplasm and uniform, round nuclei		Mitochondrial complex I genes	Loss of 1 p, loss of Y, often normal karyotype
Collecting duct	–	Partially cystic, white-gray appearance and often exhibit invasion into the renal sinus	Tubulopapillary pattern, often with cells taking columnar pattern with hobnail appearance, presence of mucinous material, desmoplastic stroma		Unknown	Losses at 8p, 16p, 1p, 9p, and gains at 13q
Medullary	–	Tan/white, poorly defined capsule, extensive hemorrhage and necrosis	Poorly differentiated, eosinophilic cells; inflammatory infiltative cells; sheet-like or reticular pattern common		Unknown	Poorly described, but believed normal karyotype
MiT family	–	Yellowish tissue often studded by hemorrhage and necrosis	Papillary or nested architecture, granular and eosinophilic cells with voluminous, cytoplasm		–	Recurrent translocations involving Xp11.2 (TFE3) or 6p21(TFEB)

Heterogeneity - Clínic

- Extension
  - Oligometastátic vs. metastátic

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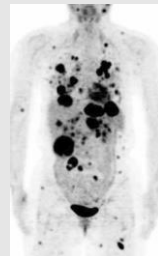
- "Timing" for dissemination
  - Metacronous < 6 m vs. Sincronous > 6 m.

Future directions

- Genétics - NGS
  - ccRCC- RECORD-3 – 261 pts
    - Somatic mutations in 341 genes.
    - With predictiv importance.
      - PBRM1 mt (41% da amostra) $\uparrow$ PFS (11,1 vs. 5,3m) com Everolimus.
      - KDM5C mt $\uparrow$ PFS (PFS 20.6 vs 8.4m) com Sunitinib.

  - Papilary RCC– 161 dts
    - Tipos 1 e 2.
    - Tipo 1 com mutações MET em 81% casos → Cabozantinib / Foretinib?
    - Tipo 2 various (3 or more subtypes)

  - Collecting Duct Ca – 17 dts
    - 36 Genétic alt. (2.1 / case)
      - NF2 (5/17, 29%) → mTORi ?
      - SETD2 (4/17, 24%)
      - SMARCB1 (3/17, 18%)
      - CDKN2A (2/17, 12%) → Palbociclib?

ANATOMIC PROFILING: THE AUTOPSY

"The Anatomy Lecture of Dr. Nicolaes Tulp" – Rembrandt, 1632

ANATOMY LECTURE
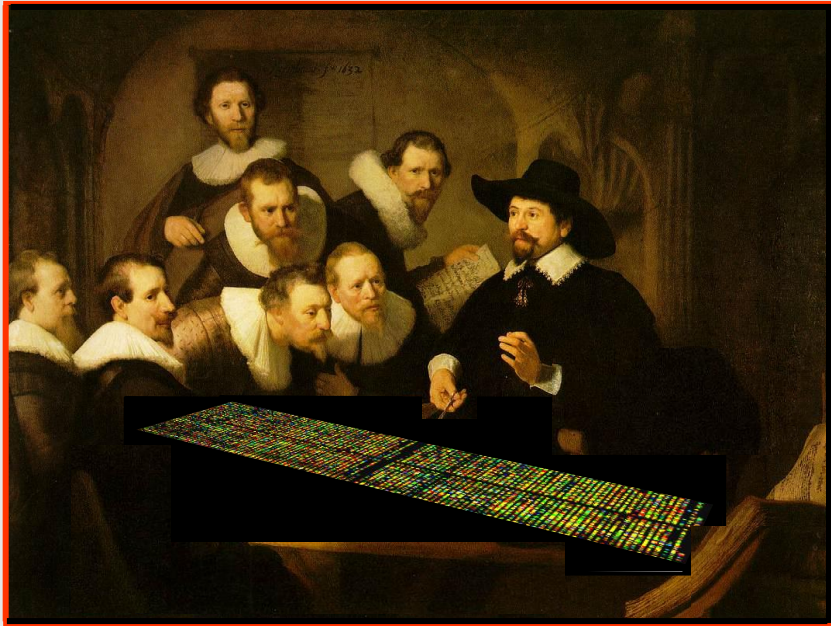
After "The Anatomy Lecture of Dr. Nicolaes Tulp" – Rembrandt, 1632

Thanks

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