RCC Classification

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http://www.utsouthwestern.edu/kidneycancer
What is the problem?
Kidney Cancer

Adapted from Linehan et al., Clin Cancer Res 2004
# Mutations in ccRCC

<table>
<thead>
<tr>
<th>Genes</th>
<th>TCGA Cohort</th>
<th>Japanese Cohort*</th>
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<tbody>
<tr>
<td></td>
<td>Tumors With Mutation (%)</td>
<td>Passenger Probability (q value)</td>
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<tr>
<td>VHL</td>
<td>52.3</td>
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<tr>
<td>PBGM1</td>
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<td>SETD2</td>
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<td>BAP1</td>
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<td>PTEN</td>
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Mutations in *BAP1* & *PBRM1* are largely mutually exclusive

\[ p = 0.00003 \]

Pena-Llopis et al., *Nat Genet* 2012

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<th>ID</th>
<th>BAP1</th>
<th>PBRM1</th>
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Pena-Llopis et al., *Nat Genet* 2012
**BAP1 and PBRM1 mutations are largely mutually exclusive in meta-analysis**

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>PBRM1</th>
<th>BAP1</th>
<th>Expected double mutants</th>
<th>p value</th>
<th>Odds Ratio (95% CI)</th>
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<tbody>
<tr>
<td>Peña-Llopis et al.</td>
<td>176</td>
<td>89</td>
<td>21</td>
<td>3</td>
<td>13 (9-16)</td>
<td>0.00003</td>
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<td>Guo et al.</td>
<td>98</td>
<td>21</td>
<td>8</td>
<td>0</td>
<td>2 (0-4)</td>
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<td>Hakimi et al.</td>
<td>185</td>
<td>53</td>
<td>10</td>
<td>1</td>
<td>3 (1-5)</td>
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<td>TCGA</td>
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<td>101</td>
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<td>10 (7-13)</td>
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<td><strong>Total</strong></td>
<td>576</td>
<td>175</td>
<td>40</td>
<td>6</td>
<td>14 (11-18)</td>
<td>0.004</td>
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</table>

Pena-Llopis et al., *Can Res* 2013
BAP1- and PBRM1-mutant tumors exhibit different gene expression signatures

TCGA: RNA-Seq for...

How many genes distinguish these random groups?

- Is this significant?
  - If instead we consider many genes would we see a similar number?
  - Is the difference between these groups significant?

- Did the same thing for...

  2,235 genes vs...
A foundation for a molecular genetic classification of ccRCC

30% wt
12% BAP1
55% PBRM1
3% BAP1/PBRM1

High grade
(q = 0.0005)

Low grade
(q = 0.025)

Pena-Llopis et al., *Nature Genet* 2012
**BAP1** and **PBRM1** genes are on chromosome 3p and one allele is frequently co-deleted with **VHL** in ccRCC.
Low grade

VHL intragenic mutation

Loss of 3p

PBRM1 mutation

BAP1 mutation

High grade

Brugarolas J. JCO 2014
May explain why $VHL^{+/−}$ humans but not $Vhl^{+/−}$ mice develop renal cancer

In the mice:

To test this hypothesis: We inactivated $Vhl$ and one allele of $Bap1$ (in nephron progenitor cells) using a Six2-Cre driver. (Loss of both copies of $Bap1$ causes renal failure and perinatal death.)

Wang et al., *PNAS* in press
Six2-Cre; Vhl \(^{F/F}\); Bap1 \(^{F/+}\) mice develop ccRCC

Wang et al., *PNAS* in press
Loss of Vhl

Loss of Bap1

Simple Cysts

Six2-Cre; Vhl^{F/F}; Bap1^{F/+}

kidneys recapitulate VHL syndrome lesions

RCC

Atypical Cysts

Payal Kapur
Low grade

High grade

Brugarolas J. JCO 2014
**BAP1** and **PBRM1** mutant tumors are associated with different outcomes

**UTSW cohort**
- HR, 2.7 (95% CI 0.99-7.6)
- Log-rank $p = 0.044$

**TCGA cohort**
- HR, 2.8 (95% CI 1.4-5.9)
- Log-rank $p = 0.004$

Kapur et al., *Lancet Oncology* 2013
Limitations of Sequencing

- Focuses on DNA.
- Uses pooled material.
- Reduced sensitivity (contamination by normal cells).
- A negative result does not guarantee normal function.
- Poor discrimination of subclonal mutations in different cell populations.
- Typically involves fresh frozen samples, which are reduced in number, and consequently has limited power for detecting mutation drivers and evaluate relationships among mutated genes.
Development of a BAP1 IHC test for broader analyses

Compared to mutation analyses:
Positive Predictive Value: ~100%
Negative Predictive Value: 98%

Pena-Llopis et al., Nat Genet 2012
BAP1 loss is associated with reduced RCC-specific survival in 1,344 patients with localized ccRCC

MAYO TEAM
Richard W. Joseph
Daniel J. Serie
Jeanette E. Eckel-Passow
Mansi Parasramka
Thai Ho
John C. Cheville
Alexander Parker

HR: 3.06; 95% CI (2.28 - 4.10)

P = 6\times 10^{-14}

Evaluation of PBRM1 by IHC in same cohort

IHC identifies tumors with simultaneous inactivation of BAP1 and PBRM1

BAP1 and PBRM1 loss in same tumor regions

BAP1 and PBRM1 loss in different tumors regions

IHC for BAP1 and PBRM1 identifies 4 molecular subtypes of ccRCC

Expected double mutants: 5.3%
Observed: 1.8%
OR, 0.18; CI 0.11-0.28, p<0.00001

Should Biology trump Nomograms?

(OR if they live the same what do I care?)

They also live the same, but are quite different!

Zigeuner et al., Eur Urol 2010
(Frank et al., J Urol 2002)
Clear cell RCC

WT               PBRM1          BAP1            BAP1/PBRM1

DRUG A           DRUG B         DRUG C           DRUG D
Conclusions

- The discovery of *BAP1* and *PBRM1* mutations in ccRCC, how they relate to each other, and how they affect outcomes establishes the foundation for the first molecular genetic and functional classification of sporadic ccRCC.

- BAP1 and PBRM1 loss define 4 distinct subtypes of ccRCC with 15-year survival rate estimates ranging from 0% to 80%.

- *BAP1*- and *PBRM1*-mutated ccRCC are associated with different: (1) histological features (grade and necrosis), (2) mTORC1 pathway activation, and (3) gene expression.

- *BAP1* is an independent predictor of outcome in patients with localized ccRCC.

Kidney Cancer

Adapted from Linehan et al., Clin Cancer Res 2004
Integrative Genomic Analyses of nccRCC
(WES, RNA-Seq and SNP CNA of 167 tumor/normal pairs)

Durinck§, Stawiski§, Pavía-Jiménez§, Modrusan§, Kapur§ et al., Nat Genet In press
DNA CNA in nccRCC

Durinck§, Stawiski§, Pavía-Jiménez§, Modrusan§, Kapur§ et al., *Nat Genet In press*
RCC classification based on top most 400 variable genes

ccRCC (TCGA)
Normal (TCGA)
Normal
pRCC
tRCC
Sarcomatoid dediff
Unclassified
Classic
Eosinopilic
Onc. Neopl. favor Eos.
RO
Onc. Neopl. favor RO

ccRCC
pRCC
Normal
RO & chRCC
chRCC
Identification of a 5-gene signature to distinguish nccRCC subtypes

Durinck§, Stawiski§, Pavía-Jiménez§, Modrusan§, Kapur§ et al., Nat Genet In press
Identified novel mechanisms of MiTF family involvement in RCC:

- Somatic translocation involving MITF
- Focal amplification of TFEB
- Indirect mechanisms of MiTF family member upregulation

Increased expression of anti-apoptotic protein BIRC7. Therapeutic target?
Brugarolas Lab
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Shannon Cohn
Haley Hill
Eboni Holloman
Meghan Konda
Fran Martinez
Andrea Pavia-Jimenez
Yifeng Gu
Renee McKay
Steven Sewell
Nick C. Wolff
Alana Christie
Xian-Jin Xie

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Nan Leng
Christian Haudenschild
Mark Ross
David Bentley

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Daniel J. Serie
Jeanette Eckel-Passow
Thai Ho
John C. Cheville
Alexander Parker

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Zora Modrusan
Sekar Seshagiri

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Vitaly Margulis
Ganesh Raj
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Eugene Frenkel

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Nathan Kim
David Pistenmaa
Robert Timmerman

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Payal Kapur
Dinesh Rakheja

Radiology
Ivan Pedrosa
Lori Watumull

Clinical Genetics
Megan Farley

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Epidemiology
Bijal Balasubramanian

Pediatric Urology
Linda Baker
Micah Jacobs
Patricio Gargollo

Pediatric Oncology
Jim Amatruda
Kenneth Chen
Jonathan Wickiser

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Dipti Ranganathan
Noelle Williams
Yang Xie
Jin Ye
Yonghao Yu