RCC: Molecular Pathways and Novel Therapies

Brugarolas Lab

James Brugarolas, M.D., Ph.D.

Kidney Cancer

I will discuss investigational use of a HIF-2 inhibitor

Research Funding: Peloton Therapeutics, Inc.

Scheuermann et al., PNAS 2009

High-Throughput Screen

Development of a HIF-2 inhibitor (HIF2-I)

HIF-2α HIF-1α Bevacizumab Sunitinib Sorafenib Pazopanib Axitinib


Scheuermann et al., Nat Chem Biol 2013

Bevacizumab

HIF-1α

HIF-1β
Testing HIF2-I in kidney cancer

Unsupervised hierarchical clustering of gene expression shows similarities between tumors and corresponding tumorgrafts

Adapted from Sivanand et al., Sci Transl Med 2012

HIF2-I is active against human ccRCC transplants in mice

HIF2-I inhibits proliferation and angiogenesis in sensitive ccRCC

Chen et al., Submitted – do not reproduce

267 mice from 22 independently derived TG lines

Chen et al., Submitted – do not reproduce
**Conclusions (Part I)**

- RCC tumorgrafts reproduce the biological properties of patient tumors.
- Inhibition of arguably the most important driver of ccRCC, the HIF-2 transcription factor, abrogates tumor growth in 56% of ccRCC tumorgrafts, including tumors resistant to sunitinib.
- HIF2-I effectively (and specifically) dissociates HIF-2\(\alpha\) from HIF-1\(\beta\) in human ccRCC implanted in mice.
- HIF-2 inhibition results in the downregulation of HIF-2 target genes and decreased circulating levels of tumor-produced VEGF.
- Primary resistance occurs despite dissociation of the HIF-2 complex in tumors.
- A Phase I clinical trial at UTSW (and elsewhere) with a first-in-class HIF-2 inhibitor has completed accrual.
Biological understanding leads to new therapies

How do we go forward?

New targets
New pathways


**PBRM1** mutations in ~50% of ccRCC.
- **PBRM1** is a two-hit tumor suppressor gene located on chromosome 3p.
- Encodes BAF180, a component of a nucleosome remodeling complex - SWI/SNF family (PBAF complex).
- Thought to regulate DNA packing and accessibility.

**BAP1** mutations in ~15% of sporadic ccRCC.
- **BAP1** is a two-hit tumor suppressor gene located on chromosome 3p and mutations abrogate protein expression.
- **BAP1** is a nuclear DUB of the UCH family implicated in cell cycle regulation, DNA replication and DNA damage repair.
- **BAP1**-mutant tumors tend to be of high grade and associated with mTOR complex 1 activation.

**BAP1** loss defines a new class of renal cell carcinoma

Pena-Llopis et al., Nat Genet 2012

What are these data telling us?

- **BAP1** is mutated in ~15% of sporadic ccRCC.
- **BAP1** is a two-hit tumor suppressor gene located on chromosome 3p and mutations abrogate protein expression.
- **BAP1** is a nuclear DUB of the UCH family implicated in cell cycle regulation, DNA replication and DNA damage repair.
- **BAP1**-mutant tumors tend to be of high grade and associated with mTOR complex 1 activation.
Under-representation of tumors with simultaneous BAP1 & PBRM1 in meta-analysis

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>PBRM1</th>
<th>BAP1</th>
<th>BAP1/ PBRM1</th>
<th>Expected double mutants</th>
<th>p value</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<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>
<td>0.10 (0.03 - 0.35)</td>
</tr>
<tr>
<td>Guo et al.</td>
<td>98</td>
<td>21</td>
<td>8</td>
<td>0</td>
<td>2 (0-4)</td>
<td>0.2</td>
<td>0.19 (0.01 - 3.43)</td>
</tr>
<tr>
<td>Hakimi et al.</td>
<td>185</td>
<td>53</td>
<td>10</td>
<td>1</td>
<td>3 (1-5)</td>
<td>0.18</td>
<td>0.23 (0.03 - 1.83)</td>
</tr>
<tr>
<td>TCGA</td>
<td>293</td>
<td>101</td>
<td>22</td>
<td>5</td>
<td>10 (7-13)</td>
<td>0.058</td>
<td>0.37 (0.14 - 1.01)</td>
</tr>
<tr>
<td>Total</td>
<td>576</td>
<td>175</td>
<td>40</td>
<td>6</td>
<td>14 (11-18)</td>
<td>0.004</td>
<td>0.29 (0.12 - 0.70)</td>
</tr>
</tbody>
</table>

A foundation for a molecular genetic classification of ccRCC

BAP1 and PBRM1 genes are on chromosome 3p and one allele is frequently co-deleted with VHL in ccRCC

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. (Loss of both copies of \( Bap1 \) causes renal failure and perinatal death).

Targeting \( Vhl \) and \( Bap1 \) in the mouse kidney causes ccRCC

Six2-Cre induces the loss of Bap1 in renal tubular cells normally expressing Bap1

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

UTSW cohort

TCGA cohort

HR, 2.7 (95% CI: 0.99-7.6)  
Log-rank p = 0.044

HR, 2.8 (95% CI: 1.4-5.9)  
Log-rank p = 0.004

Evaluation of BAP1 in 1,400 patients with resectable ccRCC from Mayo Registry

BAP1 loss is associated with reduced RCC-specific survival in the Mayo Registry

Development of a BAP1 IHC test for broader analyses

Pena-Llopis et al., Nat Genet 2012

Joseph, R.* and Kapur, P.* et al., Cancer 2013

Kapur et al., Lancet Oncology 2013
IHC identifies tumors with simultaneous inactivation of BAP1 and PBRM1

BAP1 and PBRM1 loss in different tumors regions

BAP1 and PBRM1 loss in same tumor regions

Joseph, R.* and Kapur, P.* et al., J. Urol. 2015

A foundation for the first molecular genetic classification of ccRCC

WT  PBRM1  BAP1  BAP1/PBRM1

Deadliness

The Future:

DRUG A  DRUG B  DRUG C  DRUG D

Four molecular subtypes of ccRCC with different outcomes (Mayo cohort)

Age Adjusted Risk of Death from RCC

<table>
<thead>
<tr>
<th>Genetic Profile</th>
<th>Hazard Ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBRM1- BAP1-</td>
<td>5.0 (reference)</td>
<td>0.0001</td>
</tr>
<tr>
<td>PBRM1- BAP1+</td>
<td>1.30 (0.26 - 6.89)</td>
<td>0.69</td>
</tr>
<tr>
<td>PBRM1+ BAP1-</td>
<td>3.95 (0.37 - 49.96)</td>
<td>0.43</td>
</tr>
<tr>
<td>PBRM1+ BAP1+</td>
<td>2.27 (0.66 - 8.09)</td>
<td>0.29</td>
</tr>
</tbody>
</table>

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

Joseph, R.* and Kapur, P.* et al., J. Urol. 2015

The Future:

Tumorgrafts tissue microarray:
- 58 ccRCC
  - 22 PBRM1-deficient
  - 7 BAP1-deficient
  - 3 BAP1/PBRM1-deficient

Integrated genomics (Exome, RNAseq) ~70 TG lines

TG: a platform for evaluation of BAP1 & PBRM1 pathway-targeting drugs
Conclusions (Part II)

- **BAP1** and **PBRM1** mutations define 4 subtypes of renal cancer with different biology (gene expression) and prognosis.
- These discoveries underline the foundation for the first molecular genetic classification of sporadic ccRCC.
- These findings pave the way for the development of subtype-specific treatments of previously unrecognized subtypes.
- Co-linear arrangement of multiple ccRCC two-hit tumor suppressor genes on chromosome 3p may explain human predisposition to ccRCC.
- More broadly, these findings provide a potential explanation for the differential tumor predisposition across species.