Genomic Instability & DNA Damage Response in Clear Cell Renal Cell Carcinoma

Texas Society of Clinical Oncology Annual Meeting 2017
Patrick G. Pilié, MD
Medical Oncology Fellow, MD Anderson Cancer Center
Overview

I. Background
   - VHL and ccRCC
   - DNA Damage Response (DDR) and Homologous Repair (HR)
   - Biomarker of homologous repair deficiency (HRD)

II. Patient tumor data
   - Whole exome sequencing of early stage ccRCC tumors from VHL patients.
   - TCGA KIRC for evidence of HRD score

III. Cell line models of early ccRCC
   - VHL biallelic loss alone is sufficient to cause homologous repair deficiency.

IV. Next steps
   - Targeting VHL-mutated ccRCC with DDR-directed therapies.
VHL in ccRCC

- RCC accounts for ~3% of all cancers.
  - Clear cell renal cell carcinoma is most common subtype
  - More common in men than women; most common in age>60.
- Von-Hippel Lindau gene is most common mutated gene in ccRCC. The most common genomic event in ccRCC is loss of Chr 3p.
  - Germline mutations in VHL lead to a characteristic hereditary cancer syndrome.
- Renal cell carcinoma is characterized by relatively high level of genomic instability.

Ho et al, JNCI 2014
The Von-Hippel Lindau Gene

- Located in the short arm of chromosome 3
- Tumor suppressor function
- Found in up to 80% of sporadic (non-hereditary) renal cell cancers

Adapted from Prima et al, ASCO 2006
Consequences of VHL Gene Mutation

HIF Accumulation

- VEGF, PDGFβ
- Angiogenesis

- Glut-1, Erythropoietin
- Increased Metabolism

- TGF-α, CXCR4
- Autocrine Growth Metastasis

Elongin B/C
Cul2
Rbx1
Ubiquitin Ligase Complex Disrupted

Adapted from Prima et al, ASCO 2006
ccRCC has relatively high level of genomic instability indicative of defective DNA damage response.

Adapted from Alexandrov et al, Nature, 2013
- Biallelic loss of VHL is an early event in ccRCC→engenders a mutator phenotype
- Early stage ccRCC tumors with loss of VHL have similar mutational burden to advanced ccRCC but no shared mutations, and early stage ccRCC lacks other driver mutations.
### Repair Pathways

<table>
<thead>
<tr>
<th>Repair pathway</th>
<th>NHEJ</th>
<th>HR</th>
<th>alt-NHEJ/MMEJ</th>
<th>SSA</th>
<th>ICL repair</th>
<th>SSB repair</th>
<th>BER</th>
<th>TLS</th>
<th>NER</th>
<th>MMR</th>
</tr>
</thead>
</table>

### Source of DNA Damage

- IR, radiomimetics, Topo II inhibitors
- X-linking agents, replication inhibitors, antimetabolites, Topo I inhibitors

### Damage Sensors

- Ku70/Ku80
- MRN
- PARP
- MRN

### Signaling/Mediator Proteins

- ATM, ATR, MK2, CtIP, BRCA1/BARD1, BRCA2, PALB2, RPA
- DNAPK
- CtIP

### Effector Proteins

- XRCC4, XLF, LIG4, APLF, Artemis, PAXX, WRN
- RAD51, MUS81/EME1, SLX1/SLX4, RTEL1, BLM, TOPOIII, POLQ, CtIP, POLQ, RAD52, others?
- XRCC1, LIG3, LIG1, CtiP, POLQ

### IR, ROS, radiomimetics

- Topo I inhibitors
- H₂O₂, alkylating agents

### UV, alkylating agents

- Alkylation agents, X-linkers
- DNA Pol proofreading errors

### DNA Glycosylases, APE1

- XPC, DDB2, CSA
- MSH2, MSH3, MSH6, MLH1, PMS2

### Repair Enzymes

- XPG, ERCC1, POLB, POLD1, LIG1, LIG3
- EXO1, POLD, LIG1
Cancer cells can have impaired homologous repair yet lack mutations in canonical DDR genes, such as BRCA1/2. Expression level scores can accurately identify these HR-deficient cells and provide prognostic and predictive information for solid malignancies.
Assessment of TCGA-KIRC for HR Deficiency
Peng et al, Nature Communications, 2013
TCGA-KIRC

3p gene mutation status

Distribution of mutated genes of interest

Gene: VHL, PBRM1, SETD2, BAP1, MLH1

Mutation frequency
The relationships between HR-deficiency and mutation status for the genes VHL, PBRM₁, SETD₂, and BAP₁. There were too few subjects with observed MLH₁ mutation (n=1) to allow comparison. The chi-squared test of association between HRD status and mutation status was significant at the 0.05 level for VHL, PBRM₁, and BAP₁.

KIRC TCGA database
The mosaic plot below shows the frequency of VHL mutation status (0 = no mutation, 1 = mutation) across the two classes (HR-deficient vs. HR-intact). This comparison shows that VHL mutations are more common in HR-deficient KIRC than in HR-intact (chi-squared p-value = 0.03).
The mosaic plot below shows the frequency of PBRM1 mutation status (0 = no mutation, 1 = mutation) across the two classes (HR-deficient vs. HR-intact). This comparison shows that PBRM1 mutations are more common in HR-deficient KIRC than in HR-intact (chi-squared p-value = 0.01).
Cell line models showing VHL biallelic loss engenders HR deficiency measured via DR-GFP reporter assay.
Conclusions

- Bi-allelic VHL loss with loss of chromosome 3p is an early event in renal cell carcinogenesis.
- VHL loss is sufficient to cause genomic instability via reduced homologous repair efficiency.
- VHL mutation is associated with HRD in patient samples. This may change as renal cell carcinoma progresses.
Next Steps:

- Test homologous repair efficiency using engineered cell line models with varying VHL and PBRM1 mutations to see if impact on HR efficiency is mutation dependent.
- Expand VHL-patient early ccRCC tumor set for sequencing. Evaluate RNA expression of DDR, cell cycle checkpoint, and apoptotic genes in these early stage clinical samples.
- Perform drug sensitivity and treatment studies in early ccRCC versus late ccRCC cell line models using ATR inhibitors and/or PARP inhibitors.
- Bring combination DDR-based treatments into a phase I clinical trial targeting DDR pathways in VHL-mutated tumors.
Acknowledgements

- MD Anderson Cancer Center
  - Genitourinary Oncology Department
    - Dr. Eric Jonasch, MD
    - Dr. Lijun Zhou, PhD
    - Christine Peterson, PhD
  - Cancer Prevention Department
    - Dr. Guang Peng, MD, PhD
  - Phase I Department
    - Dr. Timothy Yap, MD, PhD
  - Hematology/Oncology Fellowship Program
    - Dr. Robert Wolff
    - Co-fellows
    - Administration