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Developments in Biology of “Non-Clear Cell” RCC

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Disclosures

• None
Overview of Kidney Cancer Subtypes

- Clear cell kidney cancer represents 75% of RCC
- All the rest lumped into a basket considered “non-clear cell RCC”


2013 (ISUP Vancouver Meeting)
- Clear cell RCC
- Multilocular clear cell RCC
- Papillary I and II RCC
- Chromophobe
- Hybrid oncocytic/chromophobe
- Collecting duct
- Renal medullary carcinoma
- Neuroblastoma associated
- Mucinous tubular & spindle cell
- Tubulocystic RCC
- Acquired cystic disease RCC
- Unclassified RCC
- MiT family translocation
- HLRCC
What I did after the meeting: I ran on the treadmill with “non-Nike” sneakers, listening to music on a “non-apple” phone.....

Fifteenth International Kidney Cancer Symposium
November 4-5, 2016
Marriott Miami Biscayne Bay, Miami, Florida, USA

KidneyCancer.org
www.kidneycancersymposium.com
Rare Kidney Cancer

• The “non-clear cell designation” has led to basket trials, however limited rationale to include together in trials as subtypes have limited genetic similarities other than sharing a common organ of origin.

• A 2016 “Non-Clear Cell RCC Think Tank” recommended using the term “Rare kidney cancer” to try bring attention to each subtypes unique biology -website focused on identifying specific trials (all phases) allowing specific rare kidney cancer variants (rarekidneycancer.org)
Developments in Biology of “Rare Kidney Cancer”

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Papillary RCC: Lessons from the TCGA

Papillary Type 1

Papillary Type 2

*MET*

*FH*

Somatic Mutation Frequency

5-15%

<1%
Identification of CIMP Cluster/Warburg Class of Tumors Similar to HLRCC

- CpG Island Methylated Phenotype cluster:
- Germline FH alterations and *other* mechanisms such as CDKN2A loss, and alterations in NRF2/KEAP1 pathway
- Awful prognosis
- CIMP phenotype may be interesting population to target with inhibitors of DNA methylation, CDK4/6 inhibitors, or glycolytic inhibitors (Warburg effect)
MET Pathway: Possible alternative method causing MET upregulation?

- **MET** mutations highest incidence of mutations
  - Type 1 tumors frequency ~17% (3 germline)

- RNAseq identified a splice variant (missing exons 1&2) without extracellular ligand binding domain
- 8 tumors (~5%) found with novel MET splice variant
- In gastric cancer believed to cause ligand-independent MET signaling
Whole Genome Analysis of Papillary Renal-Cell Carcinoma (TCGA)

• Whole Genome Sequencing Analysis of 32 samples performed during TCGA at Yale and not previously reported in the NEJM Marker Paper.

• Analyses performed to assess non-coding variants that may contribute to tumorigenesis of papillary RCC
  - FunSeq developed to prioritize non-coding driver mutations
  - DELLY2 for structural variant prediction
**MET** analysis

- 6/32 (18.8%) samples carry mutations in the intronic regions between exon 1-3
- region where these is alternative splicing
- also identified 1 **MET** promoter mutation

**ERRFI1** (ERBB Receptor Feedback Inhibitor 1)
- negative regulator of EGFR family members (erlotinib has been used in pRCC)
- 6/32 (18.8%) samples have promoter mutations

**Small sample size**- unable to demonstrate expression differences

Li, Shuch, Gerstein, *Submitted for Publication*
WGS Analysis: NEAT1 Alterations

- Another mutation hotspot identified was in NEAT1, a nuclear long non-coding RNA implicated in proliferation, hypoxia, and epigenetic changes in various cancer types
- Mutations found in 5/32 (15.6%) samples were identified in a putative promoter region
- Tumors with altered cases found with higher expression and worse prognosis

Li, Shuch, Gerstein, Submitted for Publication
• WGS identifies other recurrent events that may be important in pRCC development
• Validation ongoing to verify findings in independent cohort.
Unclassified RCC

• ~4% of advanced kidney cancer considered unclassified RCC

• “Unclassified” on light microscopy does not mean specific tumors do not share common biologic classification

• Genomic characterization may shed light on biology
  - perhaps we will not identify shared morphologic features unless recognizing biology
  - ex: HLRCC features- orangiophilic nucleolus, SDH- bubbly eosinophilic cytoplasm
Genomics of Unclassified RCC

- Comprehensive analysis performed including targeted/RNA sequencing on 62 high grade tumors (~45% with metastatic disease)
- Molecular classification of these tumors may be able to find unique subsets
- Recurrent mutations:
  - NF2 (18%)
  - SETD2 (18%)
  - BAP1 (13%)
  - KMT2C (10%)
  - MTOR (8%)


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- NF2 functions like a classic tumor suppressor with LOH in samples with mutations.
- 22q12 loss was evident in the majority of cases (9/14) which carried NF2 mutations.
- Homozygous loss of chromosome 22q also was found in several samples.
- IHC expression confirms loss of protein in the NF2 altered samples.
- Leads to activation if Hippo/YAP pathway inhibition of hedgehog pathway??

Chromophobe Kidney Cancer: Role of Mitochondrial Mutations

- Both chromophobe and oncocytoma both demonstrate an abundance of mitochondria
- TCGA demonstrated that mitochondrial mutations in complex I common (mt-ND5) in ~20% of cases
- Both chromophobe and oncocytomas with tumors demonstrate >50% heteroplasmy (mixed mitochondrial genome)


mtDNA mutations and the mitochondrial phenotype?

Normal kidney cancer line exposed to inhibitor of Complex I to determine the mechanism of the mitochondrial phenotype

- Upregulation of mtDNA
- Upregulation of mt Proteins
- 1 week- extensive mitochondrial biogenesis and mitochondrial fission
- Increased mitochondrial cellular density

Shuch, et al., unpublished data
Response to Loss of Complex I in chRCC: Targeting Glycolysis?


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Conclusions

• Pathologic classification continues to be refined and we now recognize over a dozen histologic subtypes

• Rare kidney cancers (not non-clear cell RCC) account for up to 25% of the patients we see clinically

• The biology of each rare kidney cancer subtype is important and limited rationale “lumping” together with a common treatment approach
Conclusions

• The genomic basis of pRCC is very complex. MET mutations make up a small part of the type I tumors.
  - RNAseq/WGS may provide new insight into the non-MET mutant papillary RCC including splice variants and intronic/promoter alterations

• CpG Island Methylated Phenotype included the FH altered cases and loss of CDKN2A and perhaps CDK4/6 inhibition, inh. of DNA methylation, or targeting glycolysis is a rational strategy

• The inability to classify “unclassified” RCC by light microscopy is a failure of that technique.
  - ~25% of cases demonstrate dysregulated NF2/HIPPO signaling
Conclusions

• Mitochondrial mutations are common in chromophobe RCC (as well as oncocytoma)

• Loss of complex I function through mutation may lead to upregulation of mitochondrial biogenesis (by fission?) → high level heteroplasmy → provide the unique mitochondrial phenotype

  - Dysregulated metabolism/”Warburg” effect may allow therapeutic exploitation for aggressive forms of chromophobe RCC