Third Annual New Frontiers in Urology
Moffitt Cancer Center

The Panoramic View and Clinical Implications of Large Scale Kidney Cancer Global Metabolomics

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Histological Subtypes of Kidney Cancer

- **Clear Cell 70-75% (VHL)**
  - Proximal Nephron

- **Papillary Type I 5-10% (MET?)**
  - Proximal Nephron

- **Papillary Type II 5-10% (FH?)**
  - Proximal Nephron

- **Chromophobe ~5%**
  - chr (1,2,6,10,13,17)
  - Distal Nephron

- **Xp11.2 TFE3 (<1%)**
- **Medullary (<1%)**
- **Collecting Duct (<1%)**

- **Unclassified 5-10% (Heterogeneous)**

- **Others**
  - SDHB, FH, TSC1/2, BHD etc.

Hsieh, Tickoo et al
Current Signaling Targets in ccRCC

Tumor Microenvironment

Endothelial Cell

Angiogenesis

Tumor Cell

Nucleus

HIF1/2 Target Genes

RAS

PI3K

PTEN

PI3K

AKT

HIF-1/2α

TIE2

FGF

FGFR

MET

PDGFR

VEGFR

Everolimus

Temsirolimus

Sunitinib, Sorafenib, Pazopanib, Axitinib

FGFR

PDGFR

VEGFR

HIF1/2 Target Genes

S6K

4EBP1

Rheb

mTORC2

mTORC1

TSC1/2

Rheb

HIF-1/2α

Nuclear

TSC1/2

S6K

4EBP1

mTORC2

HIF-1/2α

VHL

Bevacizumab

PI3K

AKT

mTORC1

mTORC2

HIF-1/2α

VHL

Bevacizumab

PDGF

Sunitinib, Sorafenib, Pazopanib, Axitinib

VEGF
Treatment for ccRCC (Past, Now, Future)

- Sorafenib
- Sunitinib
- Temsirolimus
- High dose interleukin-2
- Interferon-\(\alpha\)
- Bevacizumab + IFN
- Axitinib
- Everolimus
- Pazopanib
- Cabozantinib
- Lenvatinib
- Nivolumab
- Pazopanib
- Nivolumab
- Ipilimumab etc.
- Precision
- Combination Sequence
- Immunization
- Prevention
- Detection

Dark Age

Modern Age

Single Arm Targeted Therapy 2005-2014

Golden Age

Hsieh, Aug 2016
Challenges of Precision Medicine

Layers of Complexity
Pan-Omics of Three Major Kidney Cancer Types

894 primary renal cell carcinomas

- mutations
- copy-number alterations
- mRNA expression
- DNA methylation
- microRNA expression
- protein expression

Clear cell-associated
Chromophobe-associated
Papillary-associated

Genomic subtypes

Percent of tumors

DNA hypermethylation
Survival correlates
Pathway alterations
Immune checkpoint

Creighton et al. Cell Reports 2016
Cancer Metabolism: Blind Men & The Elephant
Clear Cell Renal Cell Carcinoma (ccRCC, full of lipid & glycogen)
Comprehensive molecular characterization of clear cell renal cell carcinoma
Global Metabolomics Platform For Human Biospecimens

Biochemical Extraction

Instrumentation

Peak Detection
Peak Integration
Library Search
RT, Mass, MS/MS

QA/QC

In Collaboration with Metabolon
Automated Biochemical Identification

Metabolyzer Software

Mass spectrum

Biochemical ID

<table>
<thead>
<tr>
<th>Biochemical</th>
<th>Amount</th>
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<tbody>
<tr>
<td>cholesterol</td>
<td>143,789</td>
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<tr>
<td>glutamic acid</td>
<td>984,812</td>
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<tr>
<td>histidine</td>
<td>147,926</td>
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<tr>
<td>leucine</td>
<td>117,683,785</td>
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<tr>
<td>cholesterol</td>
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<td>asparagine</td>
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<tr>
<td>O-acetyl-L-carnitine-hydrochloride</td>
<td>29,443,151</td>
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<td>EDTA</td>
<td>992,513</td>
</tr>
<tr>
<td>l-aspartyl-l-phenylalanine</td>
<td>6,520,826</td>
</tr>
</tbody>
</table>
MSK TKCRP N=138 T/N Global Metabolomics Cohort

**A**

Grade
- 2 (n = 52)
- 3 (n = 67)
- 4 (n = 19)

**B**

Stage
- 1 (n = 38)
- 2 (n = 10)
- 3 (n = 70)
- 4 (n = 20)

**C**

Metastasis
- None (n = 99)
- At Presentation (n = 20)
- Recurrence (n = 19)

**D**

145 Metabolites Significantly Lower in Abundance in Tumors

129 Metabolites Significantly Higher in Abundance in Tumors

- Metabolite Type
  - Amino acid
  - Carbohydrate
  - Cofactors and vitamins
  - Energy
  - Lipid
  - Nucleotide
  - Peptide
  - Xenobiotics

- Insignificant Change

*Hakimi et al. Cancer Cell 2016*
KEGG Metabolic Pathway Analysis (T vs N)
Central Carbon Metabolism of ccRCC (Tumor versus Normal)

**Glucose**
- HK → G6P
- GPI → F6P
- ALDO → DHAP
- TPI → GAPDH
- G3P → 1,3BPG

**Glycolysis**
- F6P → FBP
- F1,6BP → 2PG
- 2PG → 3PG
- 3PG → 3PH
- 3PH → PSAT
- PSAT → 3PSE
- 3PSE → PSPH
- PSPH → GLY
- GLY → ACC
- ACC → AcCoA

**Oxidative Pentose Phosphate Pathway**
- G6PD → 6PGL
- 6PGL → 6PG
- 6PG → R15P
- R15P → RPI
- RPI → RPE
- RPE → R5P
- R5P → X5P

**Non-oxidative Pentose Phosphate Pathway**
- TALDO
- G6P
- G6PD
- 6PGL
- 6PG

**Mitochondria**
- Krebs Cycle
  - CIT
  - ACO
  - ISC
  - MAL
  - MDH
  - OAA
  - FH
  - FUM
  - SDH
  - SUC
  - SUCL
  - GLU

**Fatty Acid Synthesis**
- long chain FAs
- med. chain FAs
- Fatty Acids
- FAS
- MCoA
- ACC
- AcCoA
- ACLY

**metabolite**
- tumor vs normal
  - FC>=1.2
  - FC>=1.5
  - FC>=2

**higher in tumor**
- LAC
- PK
- PEP
- PYR

**lower in tumor**
- LDH
- ASP
- ASN

Hakimi et al. Cancer Cell 2016
Metabolic Progression of ccRCC (Supervised)

Glutathione Pathway

Dipeptides

Hakimi et al. Cancer Cell 2016
Interconnected Metabolic Progression in ccRCC

ccRCC Pathogenesis (T vs N)

Fatty Acid Synthesis
- Long chain FAs
- Medium chain FAs

Fatty Acids
- FAS
- MCoA
- AcCoA

Glutathione Metabolism
- GLU-CYS
- GCL
- GLY
- GLN
- DPEP

Polyamine and Urea Metabolism
- ARG
- UREA
- ORN
- PAOX
- ODC

Folate/Methionine Cycle
- SER
- SHMT
- GLU
- MET
- THF
- DMG
- BHMT
- Betaine
- HCYS
- AHCO
- SAH
- SAM
- DNMT

Mitochondria
- Krebs Cycle
- OAA
- MDH
- MAL
- OGDH
- IDH
- AKG
- FH
- FUM
- SDH
- SuccCoA
- SuccUCLA
- GLU
- ASP
- GOT
- ASNS
- ASN
- CIT

Higher in High Stage
- Higher in Low Stage

Hakimi et al. Cancer Cell 2016
Unsupervised NMF Consensus Clustering of ccRCC Based on Metabolites

Hakimi et al. Cancer Cell 2016
mCluster 2 (high glutathione) & mCluster 3 (high dipeptide) have worse survival (higher metastasis)
The Lack of Clear Concordance between Transcriptomics & Metabolomics: Based on Metabolic Pathways
IsoEnzymes, Allosteric Regulation, Substrate, Rx Condition

**Glycolysis**
- Glucose
- HK3, HK1, HK2
- G6P
- 6PG
- 6PGL
- PGLS
- PGD
- G6PD
- PFKP
- PFKM
- F6P
- FBP1
- TPI
- ALDO1
- GAPDH
- 1,3PG
- PGK1, PGK2
- PGM1, PGM2
- 3PG
- 3PGP
- 3PGL
- 3PGLS
- 3PPP
- 3PSER
- 2DG
- 2PG
- 2P
- ENO1, ENO2, ENO3
- PEP
- PKM2, PKL2
- PDP1, PDP2
- LACT
- LDHA
- LDHB
- LDHC
- Metabolite
- RNA
- Tumor vs normal
- FC >= 1.2
- FC >= 1.5
- FC >= 2
- Higher in tumor
- Lower in tumor

**Mitochondria**
- Krebs Cycle
- Electron Transport Chain
- NADH
- NADPH
- SUCC
- SDHC
- SDHD
- SUC
- SUC2
- SUCL2
- SUCLG1
- TCA cycle
- Oxidative phosphorylation
- ATP
- ADP
- FADH2
- NADH
- NADPH
- COX
- OGDH
- AKG
- IDH2
- ISOC
- ISC
- MDH2
- MAL
- FUM
- PEP
- DHAP
- 3PGP
- 3PGL
- 3PGLS
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**Oxidative Pentose Phosphate Pathway**
- G6P
- 6PG
- 6PGL
- PGLS
- PGD
- R1SP
- RPE
- R5P
- XSP
- G6PD
- PFKP
- PFKM
- F6P
- FBP1
- TPI
- ALDO1
- GAPDH
- 1,3PG
- PGK1, PGK2
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- R5P
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- PFKM
- F6P
- FBP1
- TPI
- ALDO1
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- 1,3PG
- PGK1, PGK2
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- 3PG
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- Lower in tumor

**RNA**
- Glucose
- NADH
- NADPH
- SUCC
- SUCL2
- SUCLG1
- SUCLG2
- TCA cycle
- Oxidative phosphorylation
- ATP
- ADP
- FADH2
- NADH
- NADPH
- COX
- OGDH
- AKG
- IDH2
- ISOC
- ISC
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**Volume 31 Number 6 June 2013 Nature Biotechnology**
Metabologram
Integrative Metabolic Analysis to Highlight Prominent Metabolic Shifts

Individual **Gene**
Fold Change

Individual **Metabolite**
Fold Change

Expression Data

Consensus **Gene**
Fold Change

Consensus **Metabolite**
Fold Change

Metabolomics Data
An Integrated Metabolic Atlas of Clear Cell Renal Cell Carcinoma
Unsupervised RNA Clusters (rCluster) Based on Metabolic Genes Expression Based on KIRC TCGA RNA-Seq Data
Hakimi et al. Cancer Cell 2016

Gene Set: Gene Ontology, Mitochondrion

-Log_{10} P Value

Log_{2} Fold Change

-1<Log_{2} FC <0
0<Log_{2} FC <1
Log_{2} FC >1

Insignificant Change

Cluster A
Cluster B
Cluster C
Cluster D

p<0.0001
Conclusion

- ccRCC exhibits broad shifts in central carbon metabolism, one carbon metabolism, and antioxidant response
- Clinical progression of ccRCC manifested with elevated glutathione and dipeptides
- Metabolomic clustering of human ccRCC identified distinct high- and low-risk subsets
- Lack of correlation between enzyme expression levels and abundance of metabolites in ccRCC
- Metabolograms visualize metabolomic, transcriptomic, and clinical data of ccRCC
- Pan-Omics including metabolomics provides integrated insights that could be exploited for prognostic and therapeutic purposes in ccRCC
Thank you &
Special Thanks to
Our Patients & Their Families