Defining and exploiting metabolic vulnerabilities in clear cell renal cell carcinoma

Edward LaGory, Ph.D.
Laboratory of Amato J. Giaccia, Ph.D.
Department of Radiation Oncology
Stanford University School of Medicine

Renal Cell Carcinoma: A Metabolic Disease

- Tumor suppressors are involved in energy sensing and metabolism.
- Clear Cell: VHL
- Does targeting ccRCC metabolism represent a viable therapeutic strategy?
  - Calithera Glutaminase inhibitors


Defining Molecular and Cellular Features of ccRCC

Constitutive Activation of HIF signaling
- Loss of VHL function results in constitutive activation of HIF pathway

Metabolic Abnormalities
- Diminished mitochondrial content and function
- Lipid droplet rich cytoplasm imparts namesake clear cell appearance

Lipid Droplets are Hubs of Lipid Metabolism and Signaling

Tun, et al. PLOS One. 2010

VHL loss induces lipid accumulation in RCC

RCC4

RCC4+VHL

Paired VHL-deficient and wild type cell lines clearly indicate that lipid droplet accumulation in ccRCC is dependent on loss of VHL

Oil Red O – Red histologic stain that labels hydrophobic lipid droplets

Lipid metabolism in renal cell carcinoma

Glucose/Glutamine → Lipogenesis

Serum Lipids → Uptake

Catabolic

Energy Production

Anabolic

Signaling/Inflammation

Membrane Biosynthesis

How does VHL-deficiency/Hypoxia regulate lipid droplet formation?

PLIN2; HIG2 - Regulation of lipid droplet stability

Cancer Discov. 2015 Jun;5(6):652-67
Sci. 2017; 8: e31132.

Suppressed fatty acid oxidation – PGC-1α, CPT-1a

LaGory et al., 2015, Cell Reports 12, 116–127


Increased Uptake – FABP3/7


Increased triglyceride synthesis – DGAT1/2


What is the source for lipid droplet formation in ccRCC?

Complete No Glutamine No Pyruvate Charcoal Stripped FBS

25mM 5mM 0mM

Glucose

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**VHL-deficiency is a key determinant of lipid droplet accumulation**

<table>
<thead>
<tr>
<th>CSFBS</th>
<th>RCC4-VHL</th>
<th>RCC4+VHL</th>
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<tr>
<td></td>
<td>50uM</td>
<td>100uM</td>
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**Oleic Acid**

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**Polyunsaturated fatty acids are susceptible to lipid peroxidation.**

- Saturated and monounsaturated fatty acids can be made via *de novo* lipogenesis while polyunsaturated fatty acids cannot.
- Polyunsaturated fatty acids are considered essential dietary nutrients.
- Linoleic acid (18:2) is the most abundant serum fatty acid.

**Implications of serum lipid metabolism on tumor biology.**

- Polyunsaturated fatty acids are susceptible to lipid peroxidation.
- Metabolism of exogenous fatty acids dictates sensitivity to ferroptosis.

- Complete FBS
- Charcoal Stripped

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**Ferroptosis – Caspase-independence programmed cell death mediated by lipid peroxidation**


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**Metabolism of exogenous fatty acids dictates sensitivity to ferroptosis**


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Unsaturated fatty acids drive sensitivity to Erastin-induced ferroptosis

Acyl CoA Synthetases (ACSL) activate exogenous fatty acids for downstream metabolism

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Are specific ACSL isoforms required for lipid droplet accumulation in RCC?

ACTS in Lipoic Acid

Lipid Biosynthesis

Circulating Fatty Acids

R-C-OH

ATP

AMP+PP

R-C-S-CoA

Lipid Biosynthesis

Circulating Fatty Acids

R-C-OH

ATP

AMP+PP

R-C-S-CoA

Lipid Biosynthesis
ACSL inhibition protects against Erastin-induced ferroptosis

ACSL inhibition kills ccRCC cell lines through a caspase-dependent mechanism.

Genetic suppression of ACSL3 reduces viability in ccRCC cell lines

Working Model

- Exogenous fatty acid metabolism drives lipid droplet formation in VHL-deficient cells.
- Metabolism of polyunsaturated fatty acids imparts sensitivity to ferroptosis.
- ACSL3 is a critical driver of lipid droplet formation in ccRCC.
- ACSL inhibition leads to caspase-dependent cell death.
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ACSL family gene expression in ccRCC

- ACSL5 expression is increased in ccRCC tumors compared to normal kidney.
- Increased ACSL5 expression in tumors occurs independently of tumor stage
- ACSL6 not expressed at detectable levels

ACSL3 is required for lipid droplet accumulation in ccRCC

Verification of “on target” effects of ACSL3 siRNA
SmartPool

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Deconvolution of siRNA SmartPool by investigating the effect of individual siRNAs

Genetic suppression of ACSL1 or ACSL3 reduces viability in ccRCC cell lines

Lipid droplets may defend against lipid peroxidation

Triacsin C treatment induces accumulation of 4-HNE