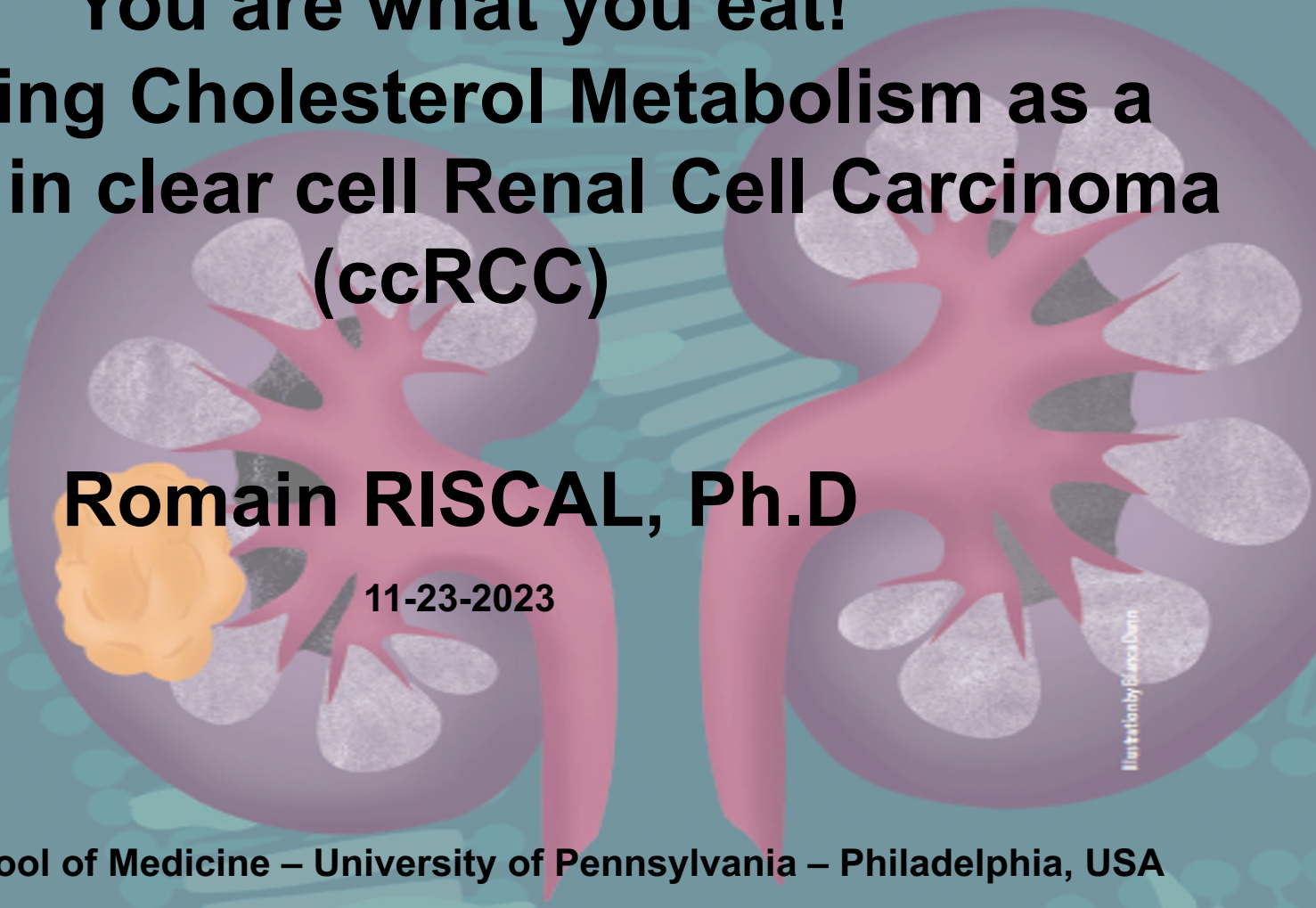
5TH EDITION METABOLISM & CANCER

You are what you eat! Targeting Cholesterol Metabolism as a Liability in clear cell Renal Cell Carcinoma (ccRCC)

Romain RISCAL, Ph.D

11-23-2023



Perelman School of Medicine – University of Pennsylvania – Philadelphia, USA

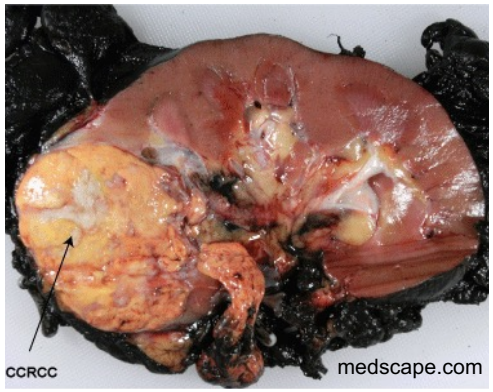
Kidney Cancer at a Glance

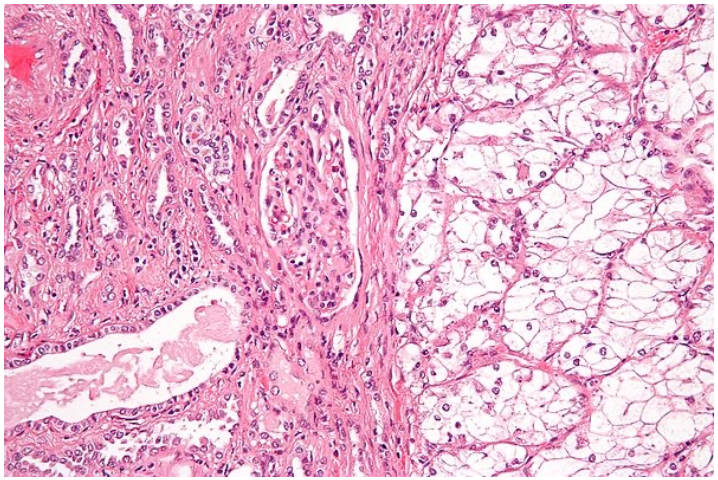
- **Clear Cell Renal Cell Carcinoma (ccRCC)** is the predominant subtypes of Kidney cancer.

  - **Clear cell renal cell carcinoma** (**ccRCC: 70-75%**)
  - Papillary (10%)
  - Chromophobe (5%)
  - Cystic-solid (1-4%)
  - Collecting ducts (1%)
  - Medullary (<1%)
  - Xp11 translocation (<1%)
  - Mucinous tubular and spindle cell (<1%)
  - Associated with neuroblastoma (<1%)
  - Non-classified (4-6%).

- **ccRCC genetic alteration (>90%)**: 3p deletion or translocation (***VHL***), leading to HIF stabilization.

- **ccRCC morphology**: "clear cell" due to lipids and glycogen accumulation.

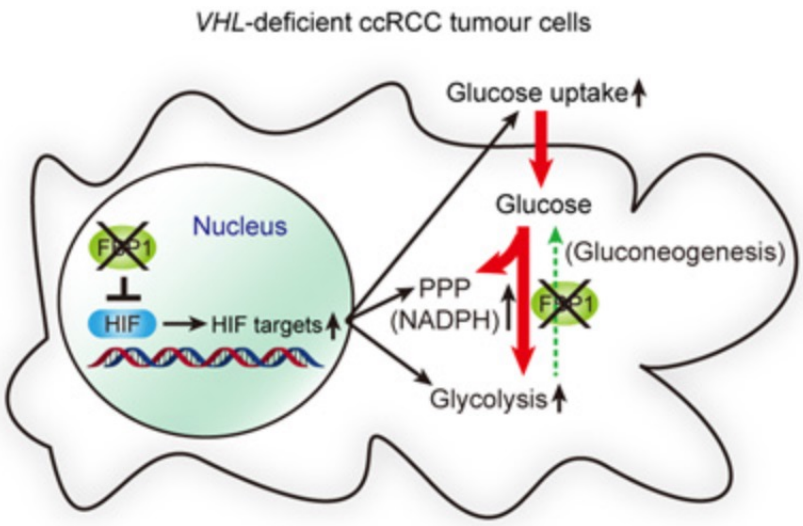




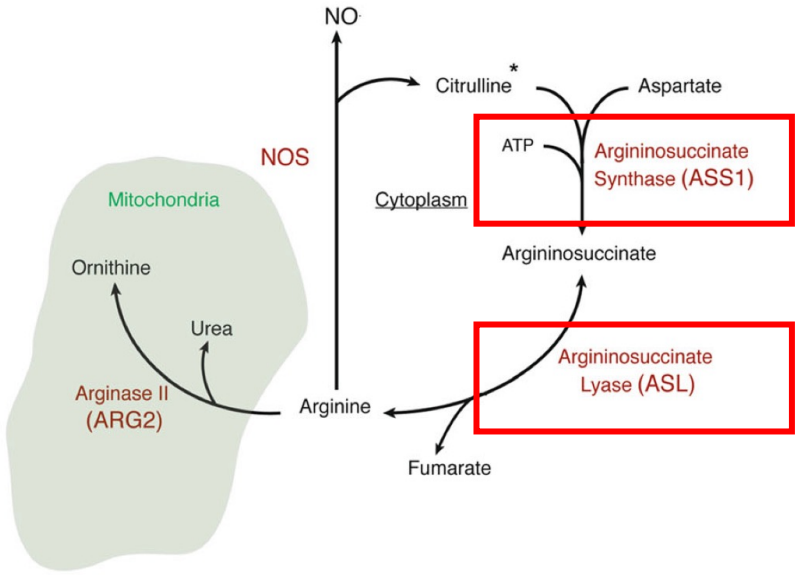
Medscape.com

Normal Tissue **ccRCC**

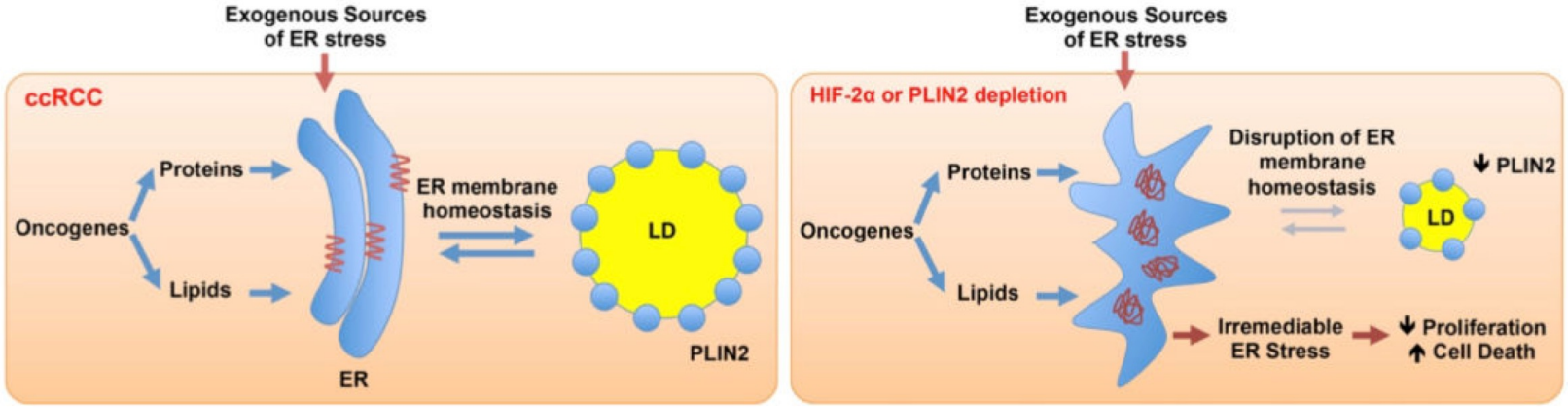
Deregulated Metabolism: Hallmark of ccRCC



Bo Li et al., Nature. 2014

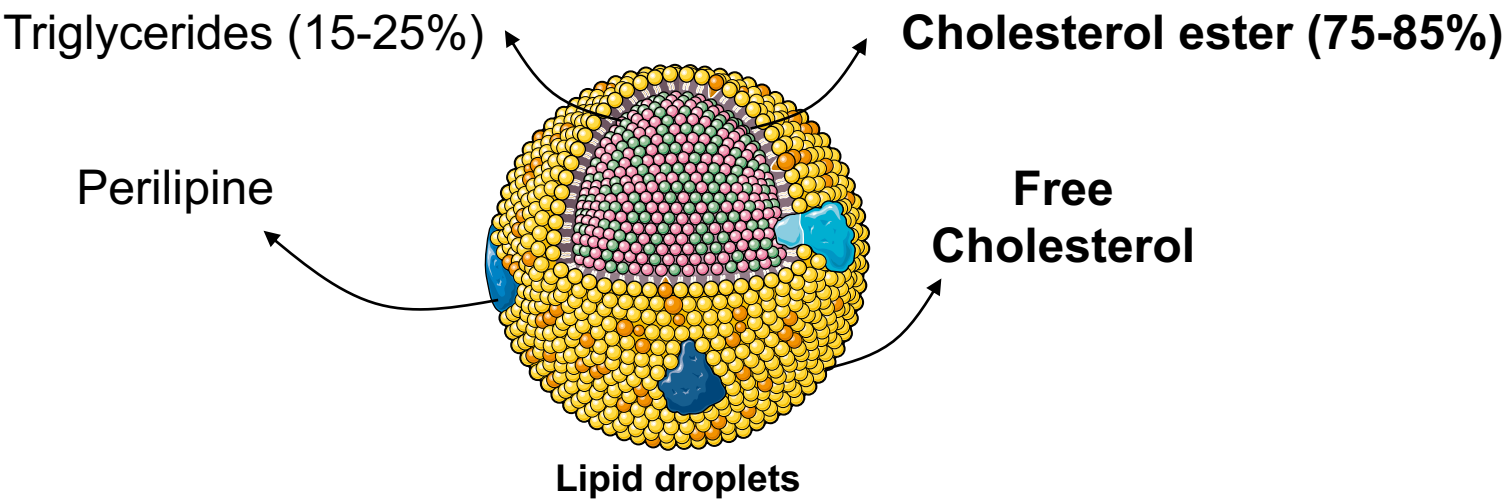


Khare et al., Cancer & Metabolism. 2021

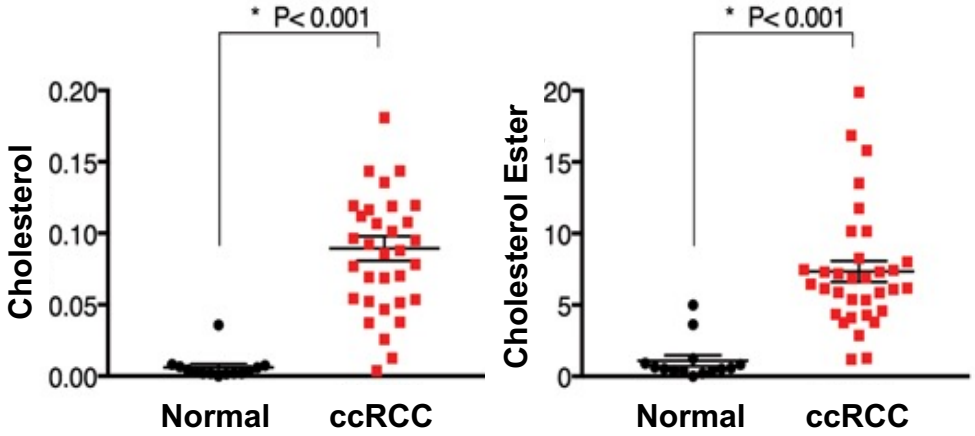


Bo Qiu et al., Cancer Discovery. 2015

ccRCC Phenotype: Lipid Droplets (LD) & Glycogen

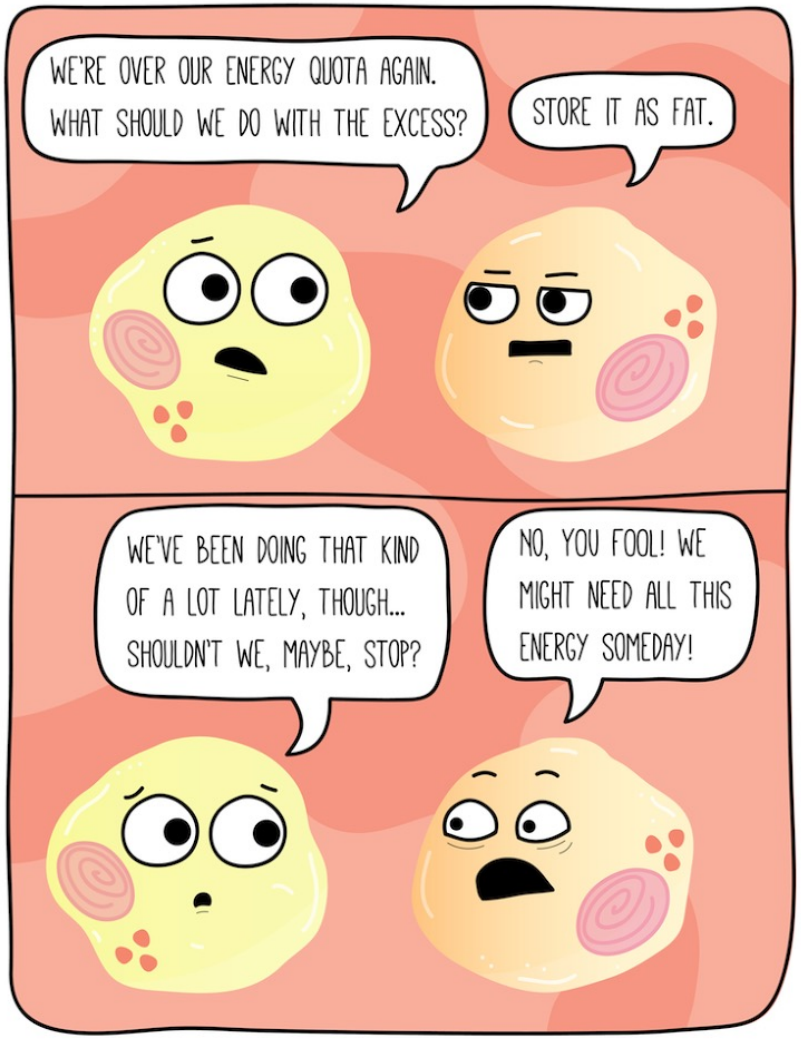


- **Roles**: sources of lipids, energy storage, avoid toxicity…
- **High Cholesterol** (3-8x) and **Cholesterol Ester** (20/100x) levels.



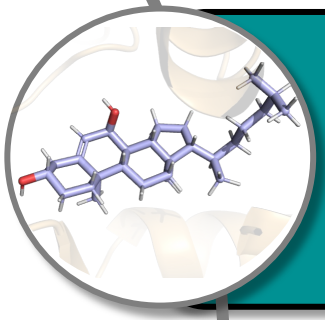
Zhang et al., JCI. 2017

Does excess cholesterol contribute to ccRCC?

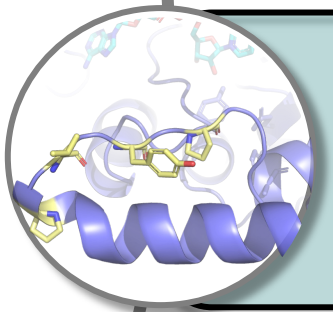


Beatrice the Biologist

Targeting Cholesterol Metabolism?

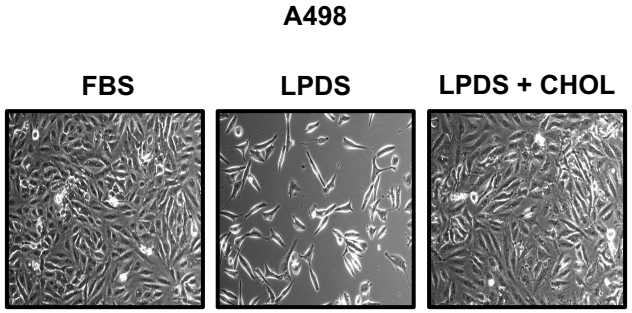


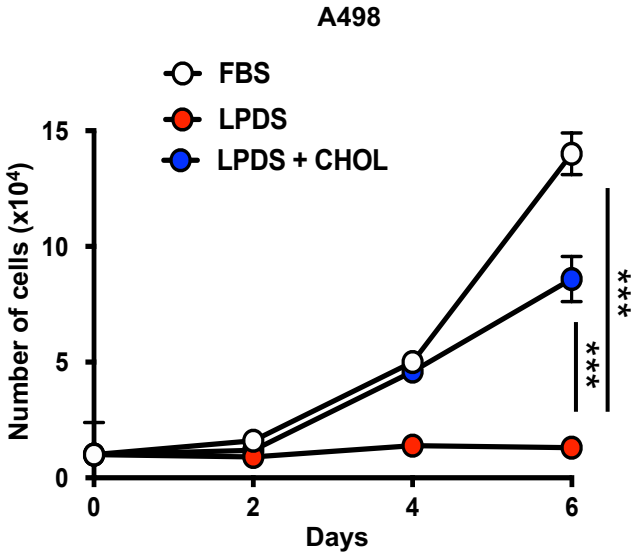
Cholesterol Auxotrophy as a Targetable Vulnerability in Clear Cell Renal Cell Carcinoma

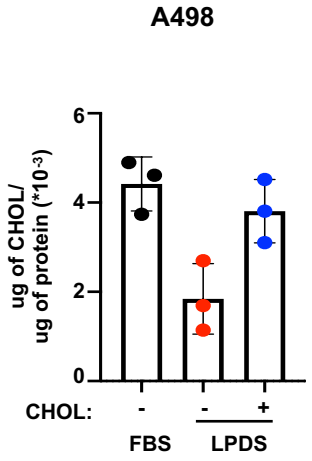


Re-activation of the Bile Acid Pathway Confers Growth Advantages to ccRCC

Exogenous Cholesterol is Essential to Mediate ccRCC Cells Proliferation *in vitro*

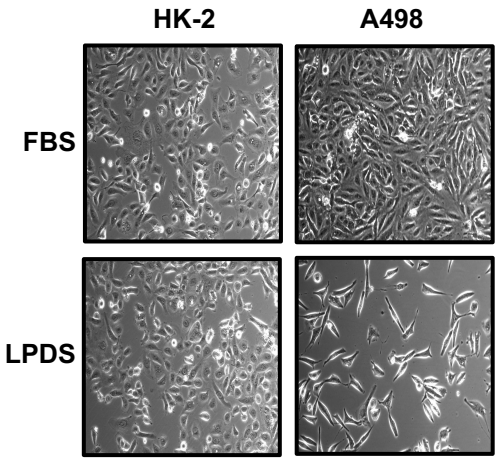


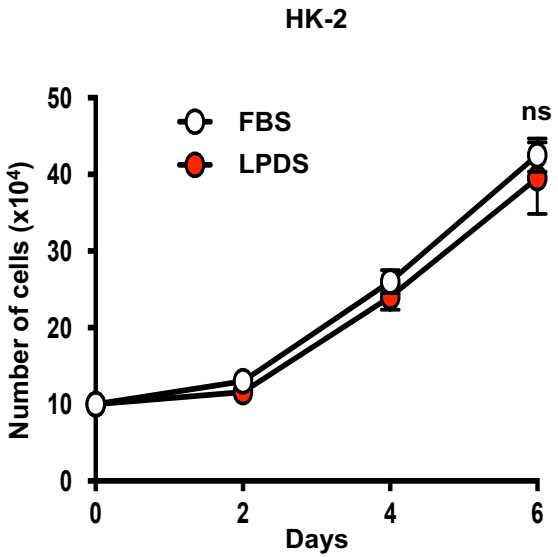


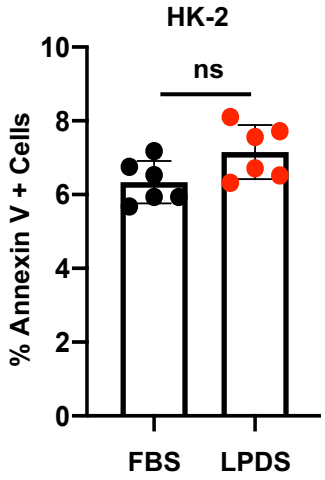


Riscal R. et al., Cancer Discovery. 2021

Normal Kidney Cells are not Affected by Exogenous Lipid Deprivation

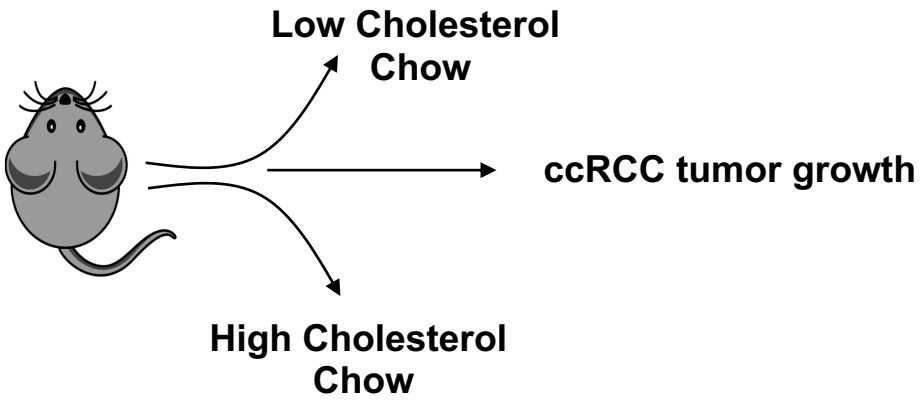


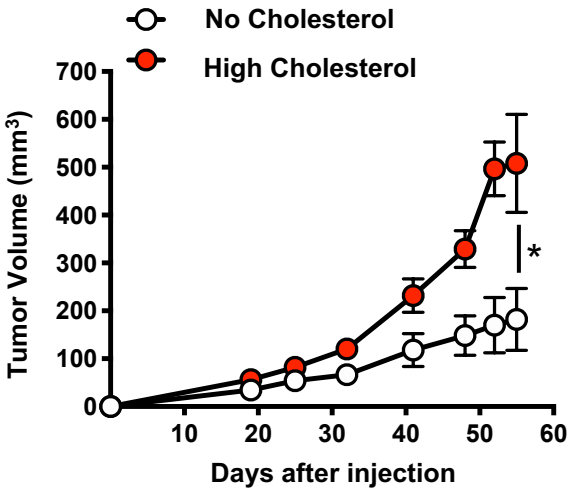


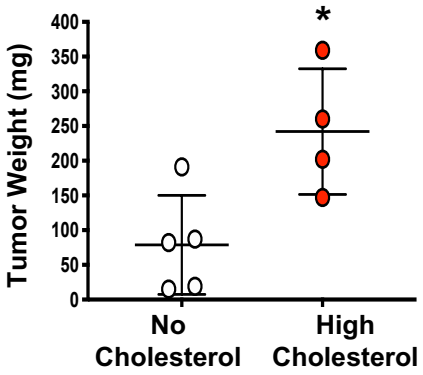


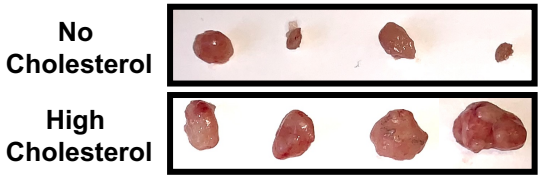
Riscal R. et al., Cancer Discovery. 2021

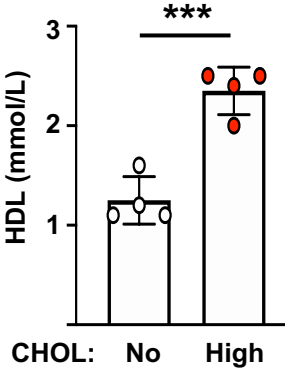
Consequence of Cholesterol deprivation on ccRCC xenografts





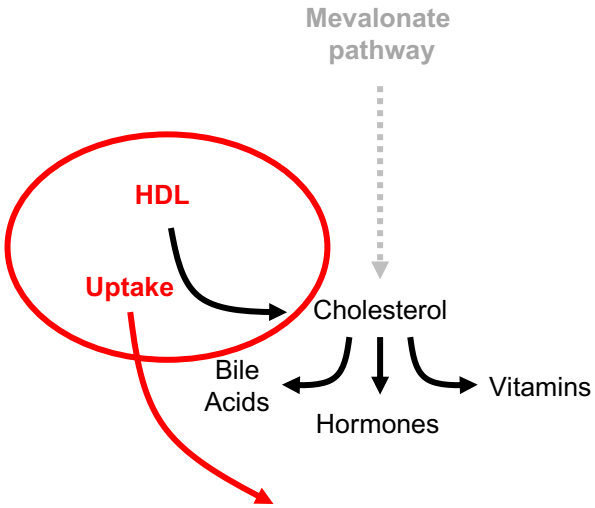






Riscal R. et al., Cancer Discovery. 2021

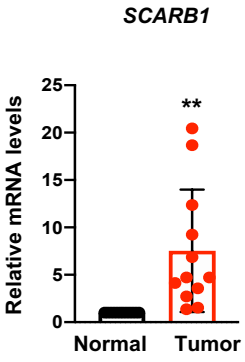
How ccRCC cells end up loaded with high cholesterol content

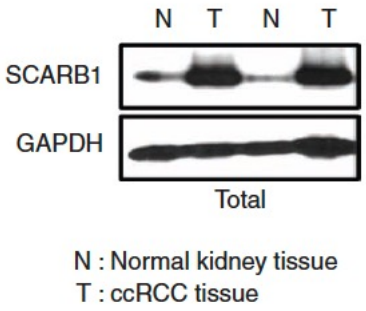


- **Mainly expressed in the liver for Reverse Cholesterol Transport (RCT)**
- **Uptake of Cholesterol Ester from HDL**
- **Cholesterol efflux**
- **Involved in viral infection (HBV & COVID19)**

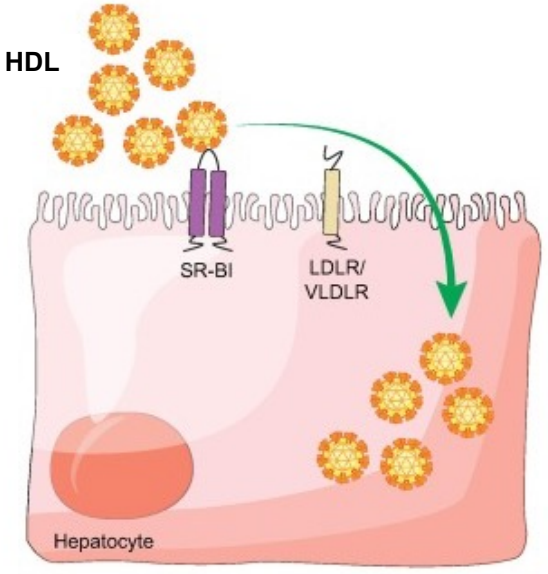
Gene involved in Cholesterol uptake

Gene	Gene expression changes in tumor vs normal tissue	p-adj
SCARB1	16.80653	4.66E-208
LDLR	-2.39152	9.65E-14
VLDLR	1.55408	4.29E-11
CD36	2.16743	3.87E-44



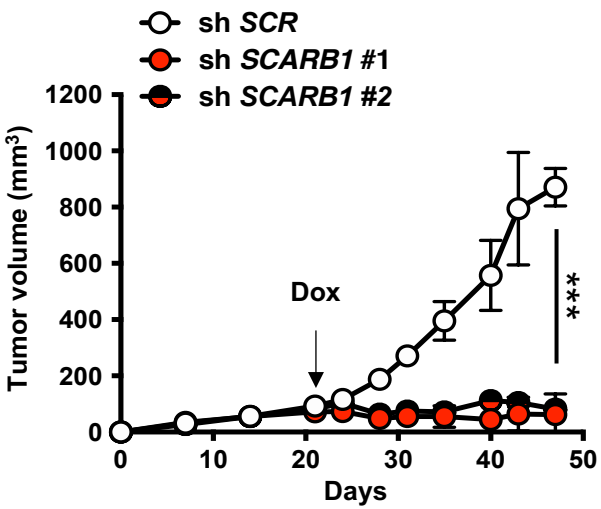


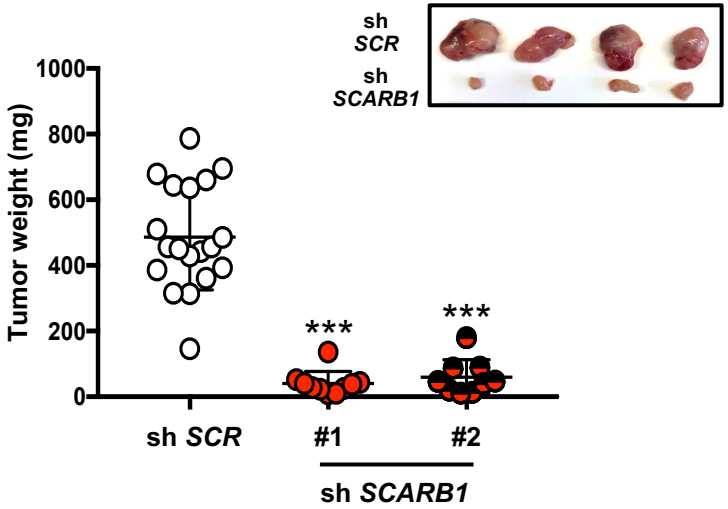
N : Normal kidney tissue
T : ccRCC tissue



Riscal R. et al., Cancer Discovery. 2021

Targeting *SCARB1* impairs Tumor Growth *in vivo*





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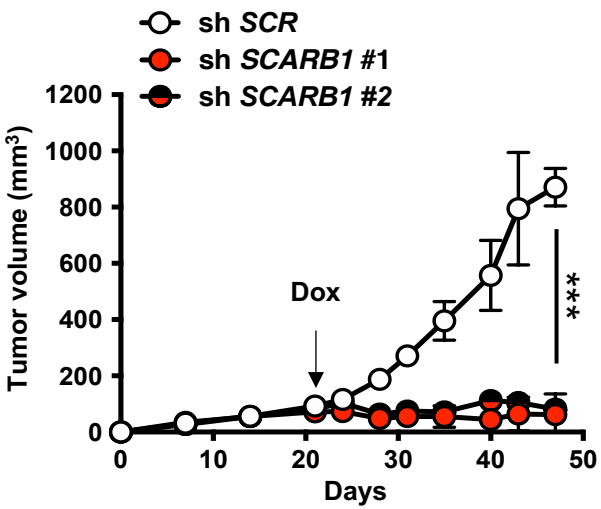
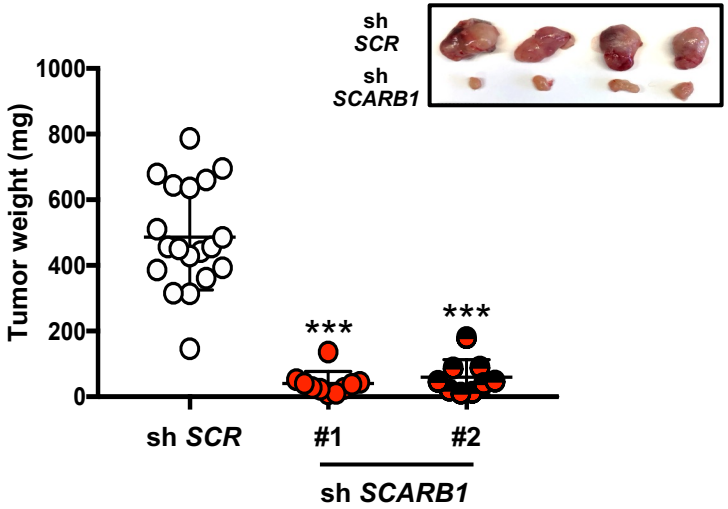
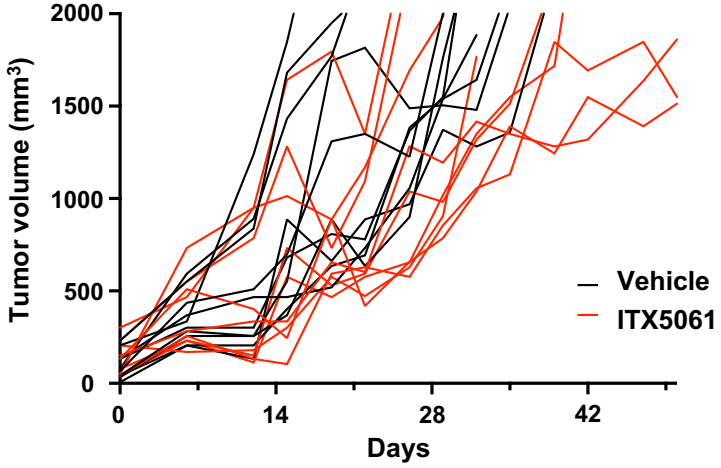
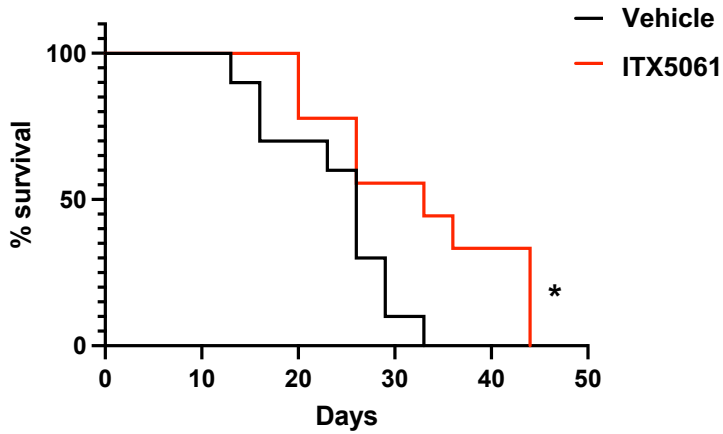




Riscal R. et al., Cancer Discovery. 2021

ccRCC PDXs





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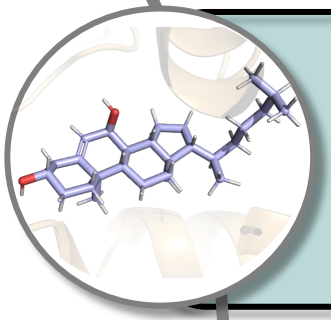
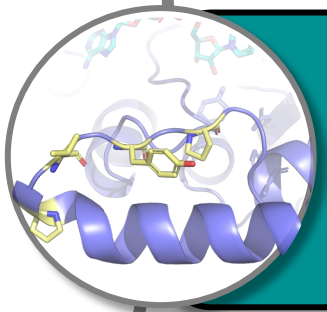


Cholesterol Auxotrophy as a Targetable Vulnerability in Clear Cell Renal Cell Carcinoma



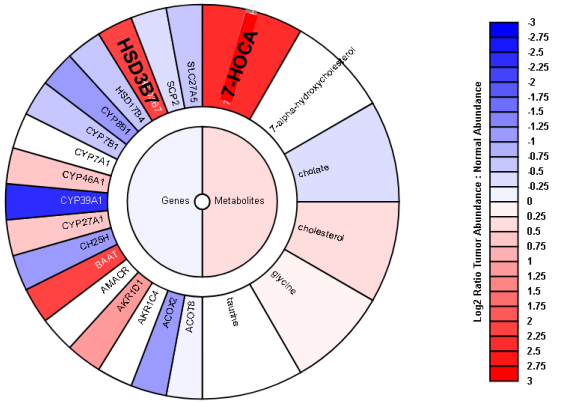
Re-activation of the Bile Acid Pathway Confers Growth Advantages to ccRCC

Bile Acid Linked with ccRCC Patients

Metabolite	δ_H ppm[a] (multiplicity)	% variation (±uncertainty)	ES (±ES_{SE})	*p*-value
Metabolite variations in controls > 60 yr (n = 29) *vs.* controls ≤ 60 yr (n = 20)				
2-KG[b]	2.45 (t)	74.3 (6.1)	2.08 (0.52)	1.47×10^{-12}
2-Py	8.33 (s)	32.3 (12.2)	0.54 (0.43)	8.15×10^{-3}
3-methylhistidine[b]	8.11 (s)	4.9 (13.3)	0.08 (0.42)	
3-HIBA[b]	1.36 (s)	4.7 (5.6)	0.18 (0.42)	
3-HIVA	2.37 (s)	3.0 (5.1)	0.13 (0.42)	
4-DTA	1.24 (d)	−19.9 (11.0)	−0.41 (0.43)	
4-hydroxyhippurate	7.76 (d)	−51.3 (11.3)	−1.17 (0.45)	2.95×10^{-9}
4-hydroxyphenylacetate[b]	6.88 (d)	−13.9 (6.6)	−0.47 (0.32)	5.09×10^{-3}
Acetate	1.93 (s)	−63.2 (37.2)	−0.45 (0.43)	
Acetone	2.24 (s)	−6.6 (7.7)	−0.21 (0.42)	1.54×10^{-4}
Allantoin[c]	5.40 (s)	6.4 (7.2)	0.19 (0.42)	
Ascorbate	4.53 (d)	−14.4 (7.0)	−0.47 (0.43)	
Bile acid[†]	0.54 (s)	75.5 (13.7)	0.92 (0.44)	5.51×10^{-6}
Bile acid[†]	0.57 (s)	101.0 (15.2)	1.04 (0.45)	1.61×10^{-7}

Monteiro et al., Scientific reports, 2016

Primary Bile Acids Biosynthesis



Hakimi et al., Cancer Cell, 2016

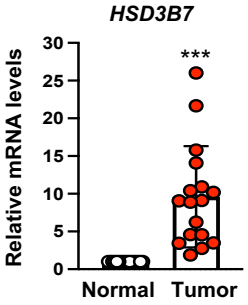
Classical pathway

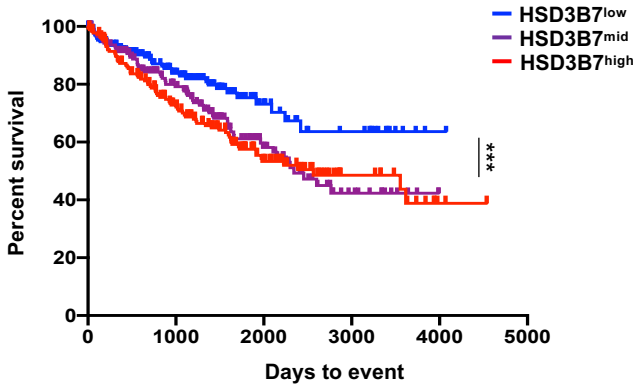
Cholesterol
ROS
CYP7A1
7α hydroxycholesterol
HSD3B7
7α-hydroxy-3 oxo 4-cholestenic acid
CYP8B1
5β-cholestan-3α,7α,12α-triol
5β-cholestan-3α,7α-diol
CYP27A1
3α,7α-dihydroxy-5β cholestanoic acid
Cholic Acid
Chenodeoxycholic Acid

Alternative pathway

Cholesterol
CYP27A1
27 hydroxycholesterol
CYP7B1

HSD3B7, a Promising Target in ccRCC





Riscal et al. in Revision

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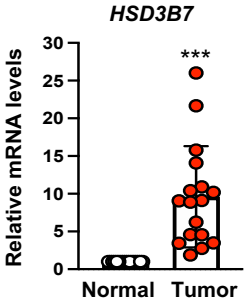
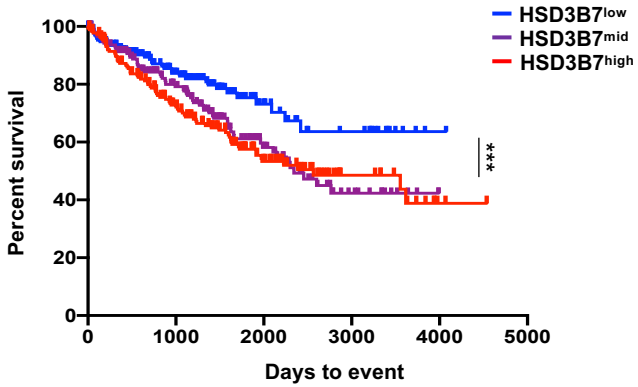
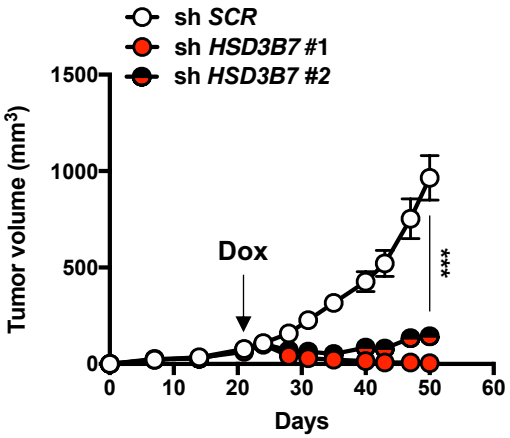
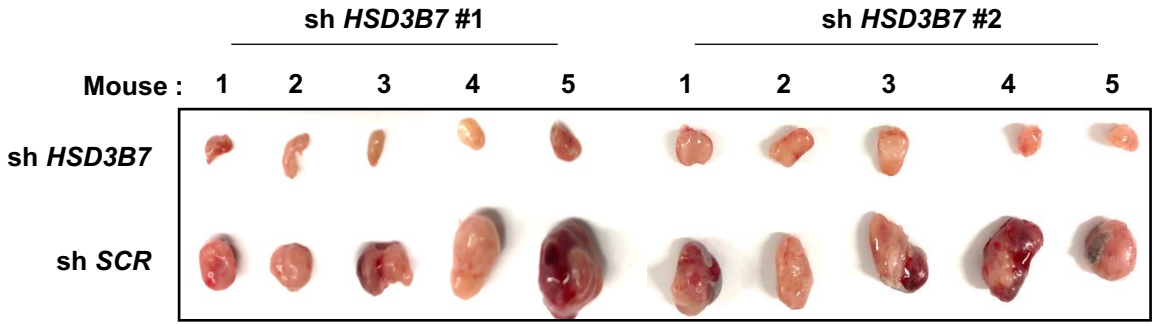
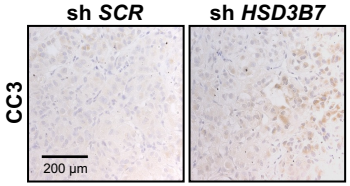
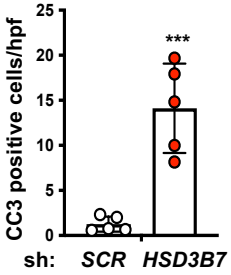






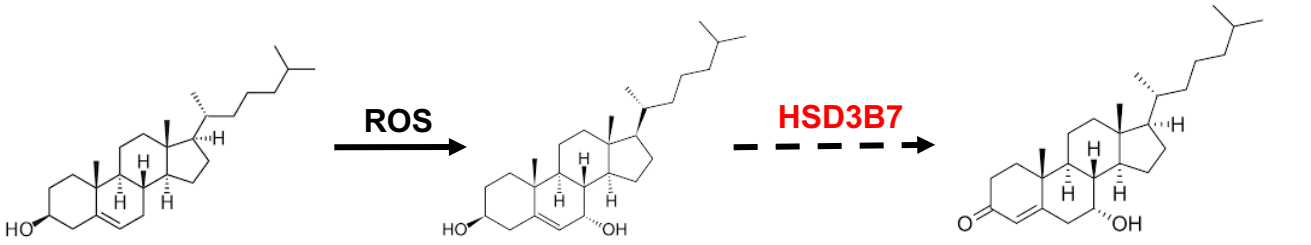






Riscal et al. in Revision

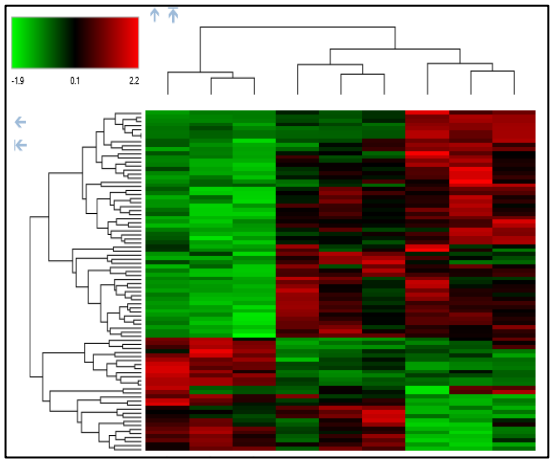
HSD3B7 Knock Down Lead to 7α-OHC Accumulation



Cholesterol
0.095 mg/L

7 alpha-hydroxycholesterol (7α-OHC)
0.48 mg/L

7α-hydroxy-3-oxo4-cholestanoic acid (7-HOCA)
3.7 mg/L



sh *SCR* *#1* *#2*

sh *HSD3B7*

Riscal et al. in Revision

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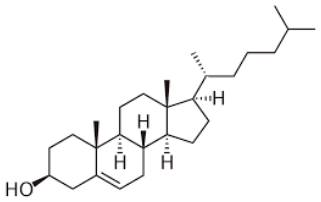
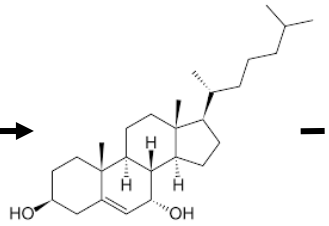


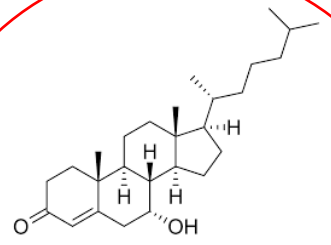
Cholesterol
0.095 mg/L

ROS

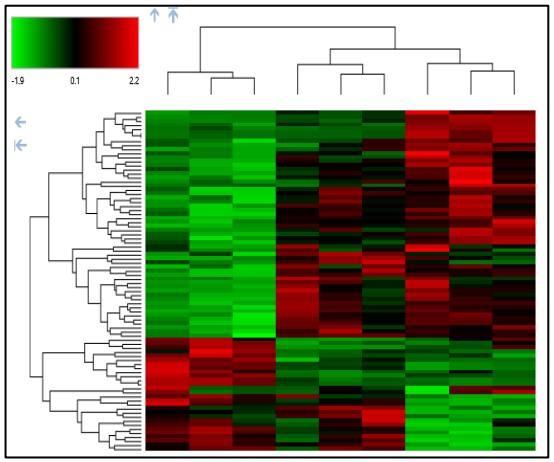


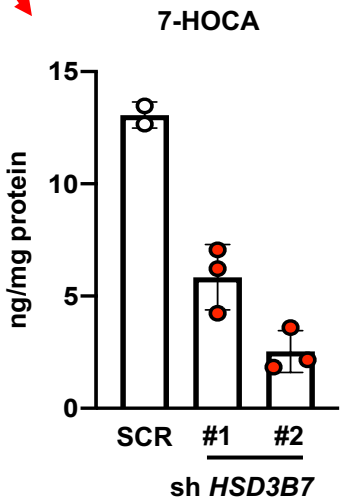
7 alpha-hydroxycholesterol (7α-OHC)
0.48 mg/L

HSD3B7



7α-hydroxy-3-oxo4-cholestanoic acid (7-HOCA)
3.7 mg/L





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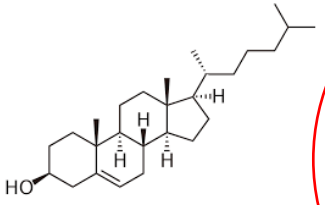
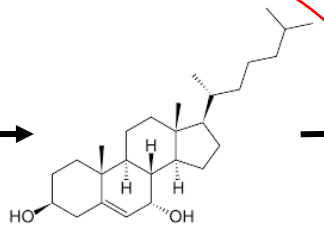
HSD3B7 Knock Down Lead to 7α-OHC Accumulation



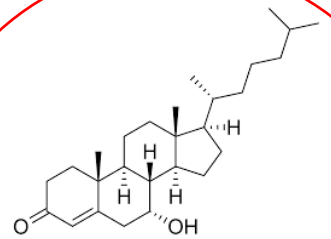
Cholesterol
0.095 mg/L

ROS

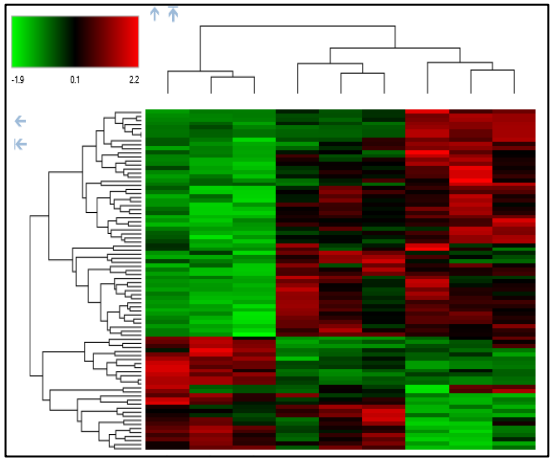


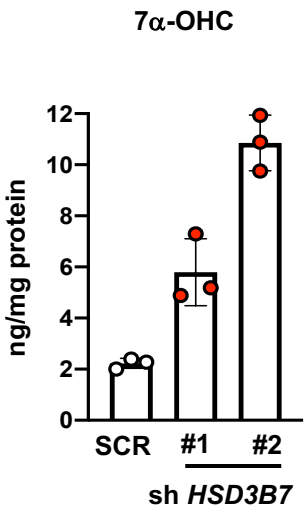
7 alpha-hydroxycholesterol (7α-OHC)
0.48 mg/L

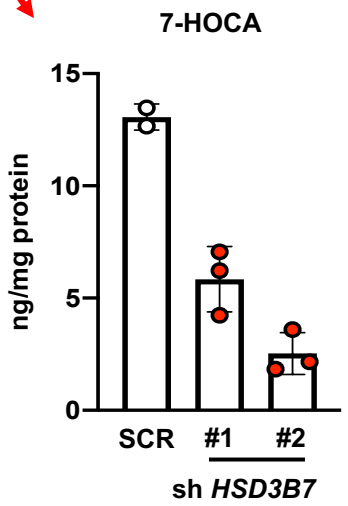
HSD3B7



7α-hydroxy-3-oxo4-cholestanoic acid (7-HOCA)
3.7 mg/L







Is accumulation of oxysterols toxic for ccRCC cells ?

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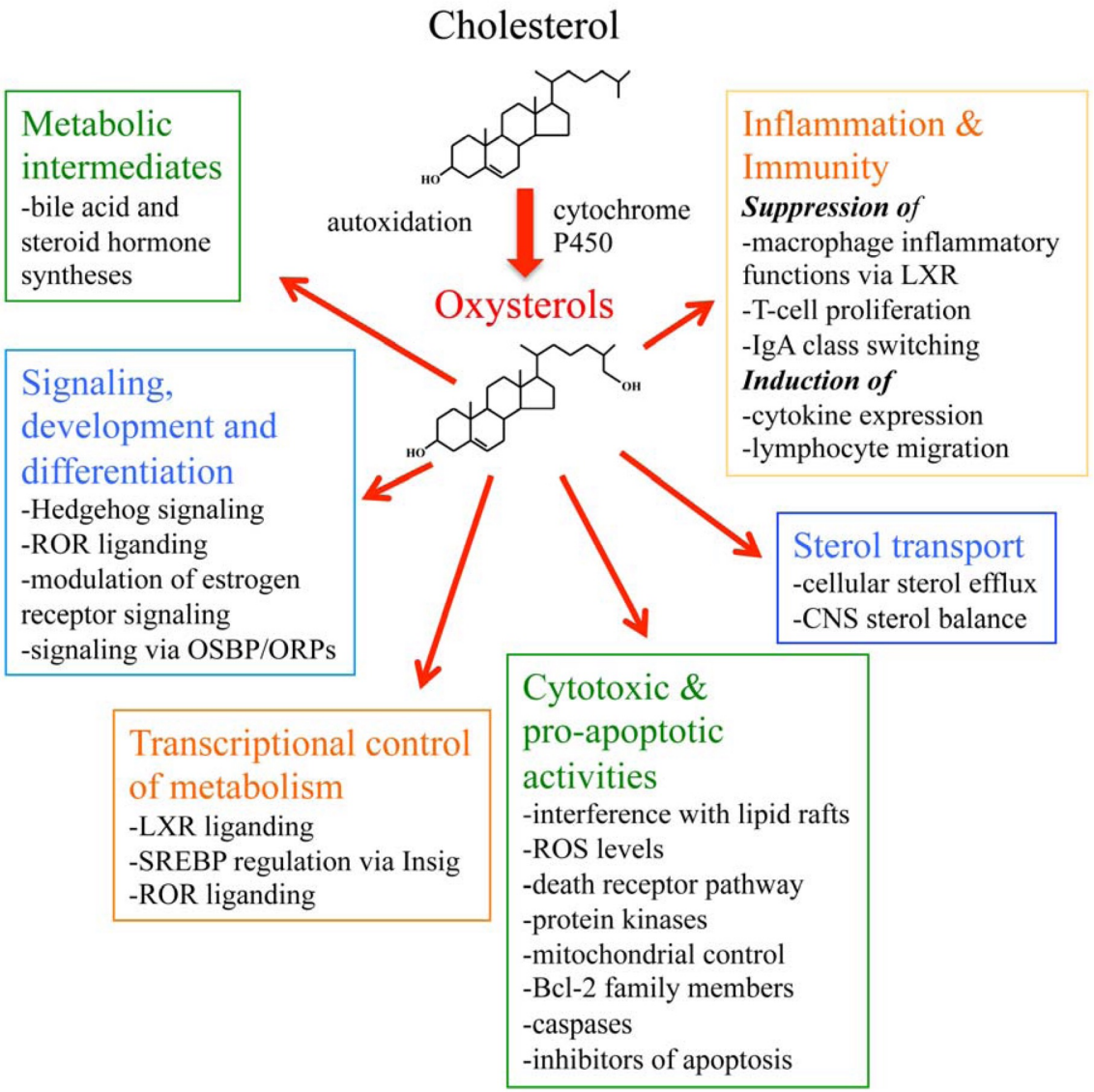
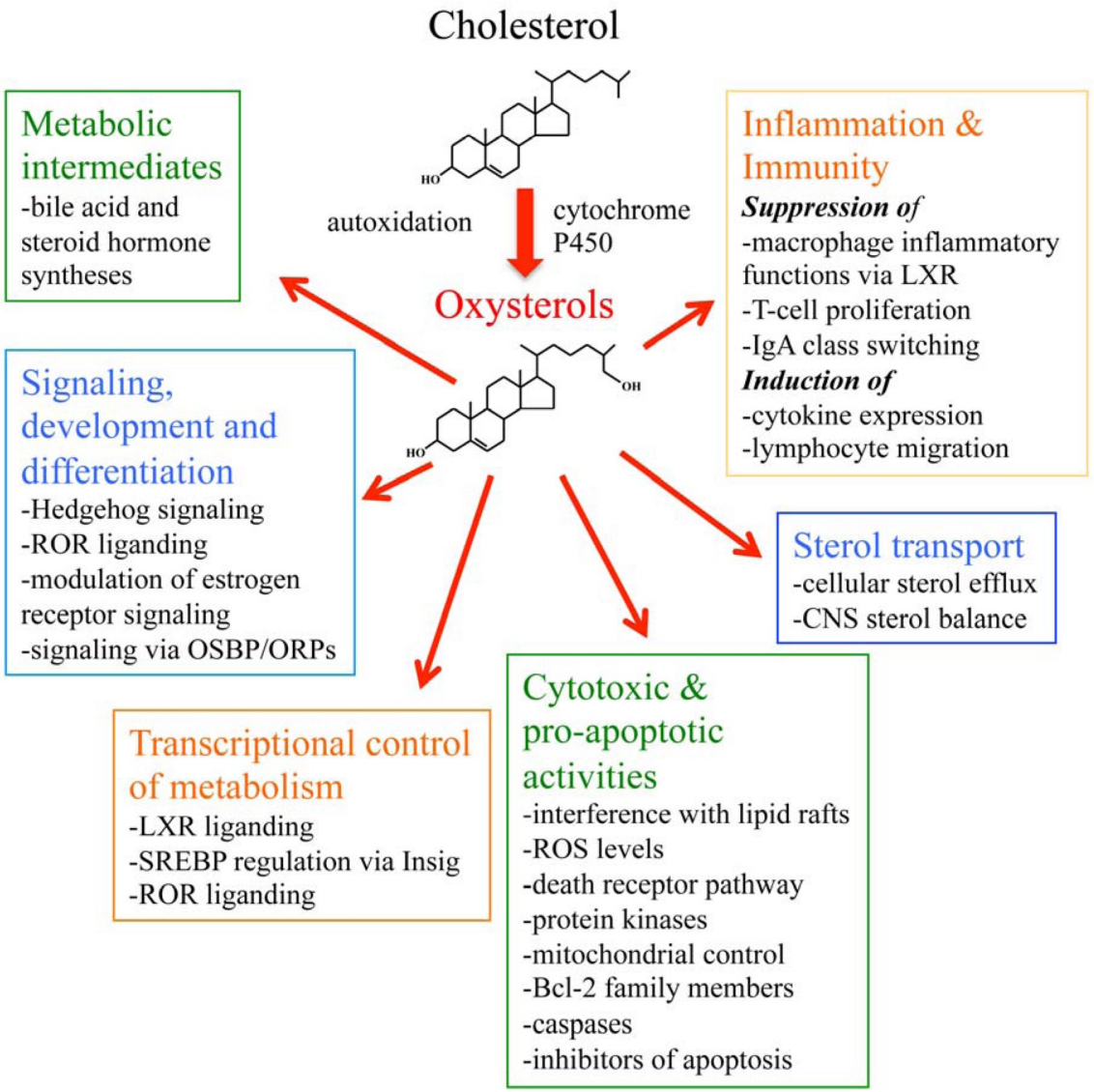
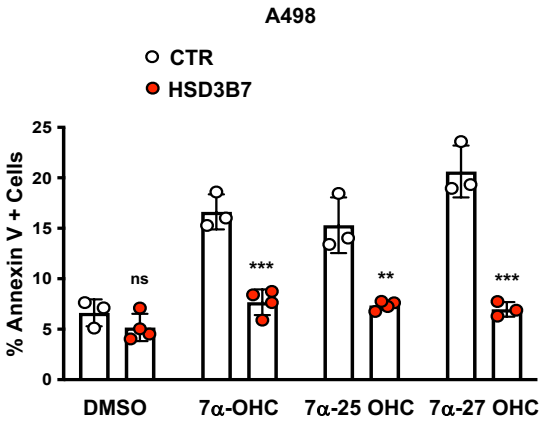
7α-OHC Accumulation Induces ccRCC Cell Death

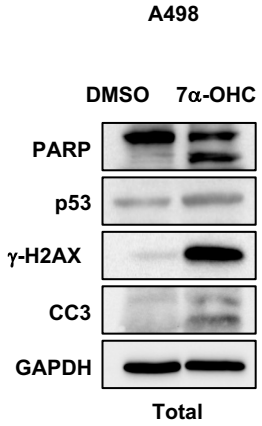


Olkkonen VM. et al., Biomolecules. 2012

7α-OHC Accumulation Induces ccRCC Cell Death







Olkkonen VM. et al., Biomolecules. 2012

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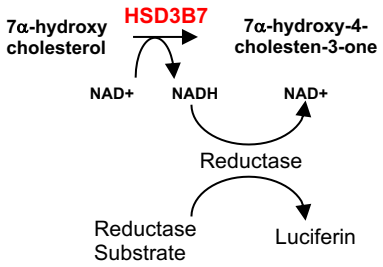
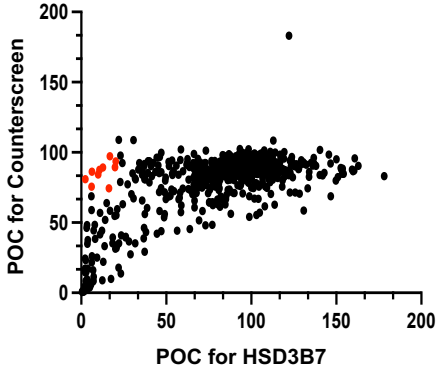
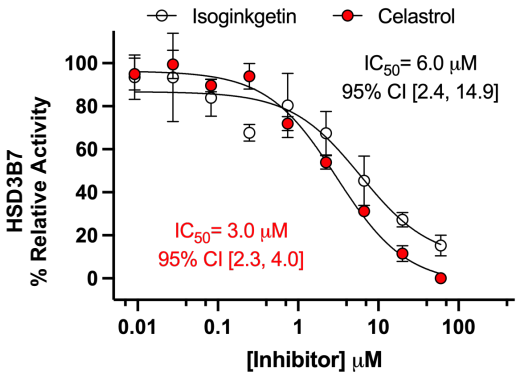
Natural Library Inhibitor Screening Reveals Celastrol as a Potent HSD3B7 Inhibitor

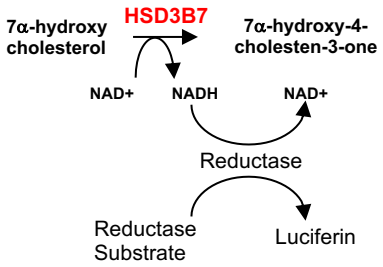


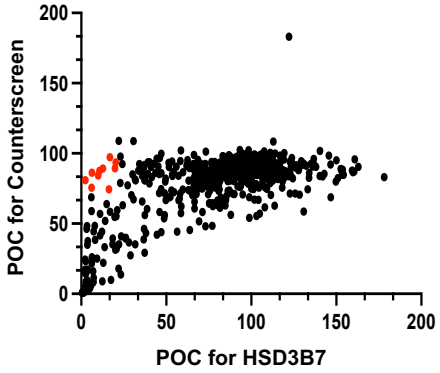


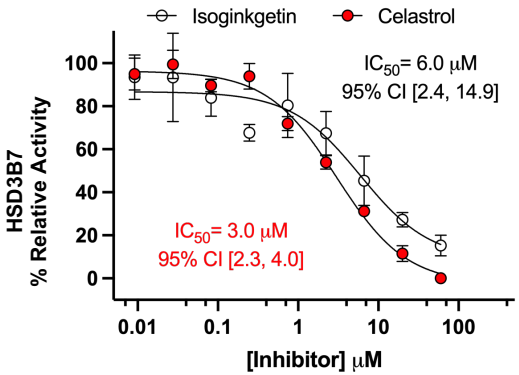


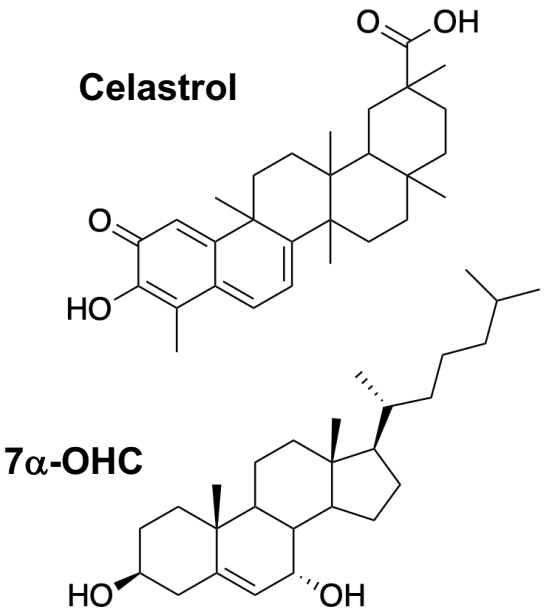
Riscal et al. in Revision

Natural Library Inhibitor Screening Reveals Celastrol as a Potent HSD3B7 Inhibitor









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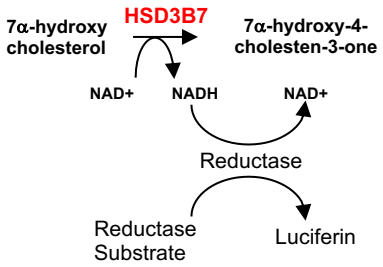
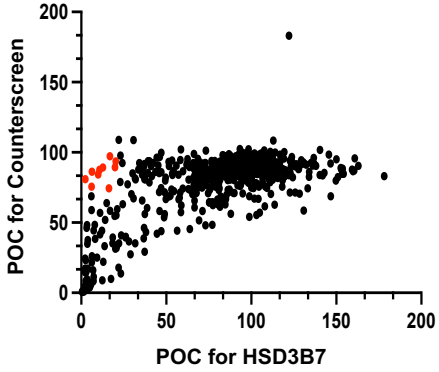
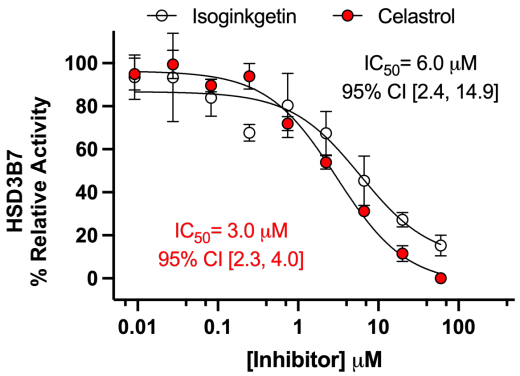
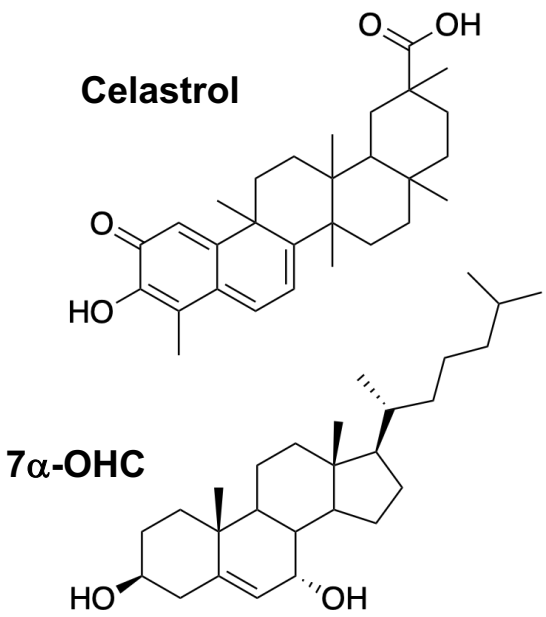
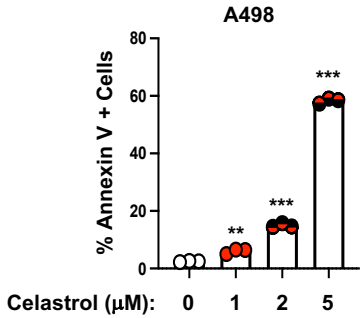
Natural Library Inhibitor Screening Reveals Celastrol as a Potent HSD3B7 Inhibitor

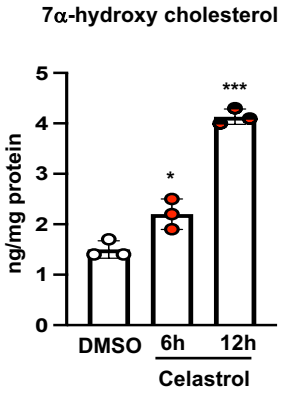


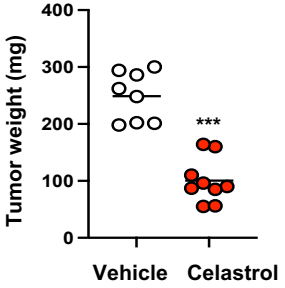












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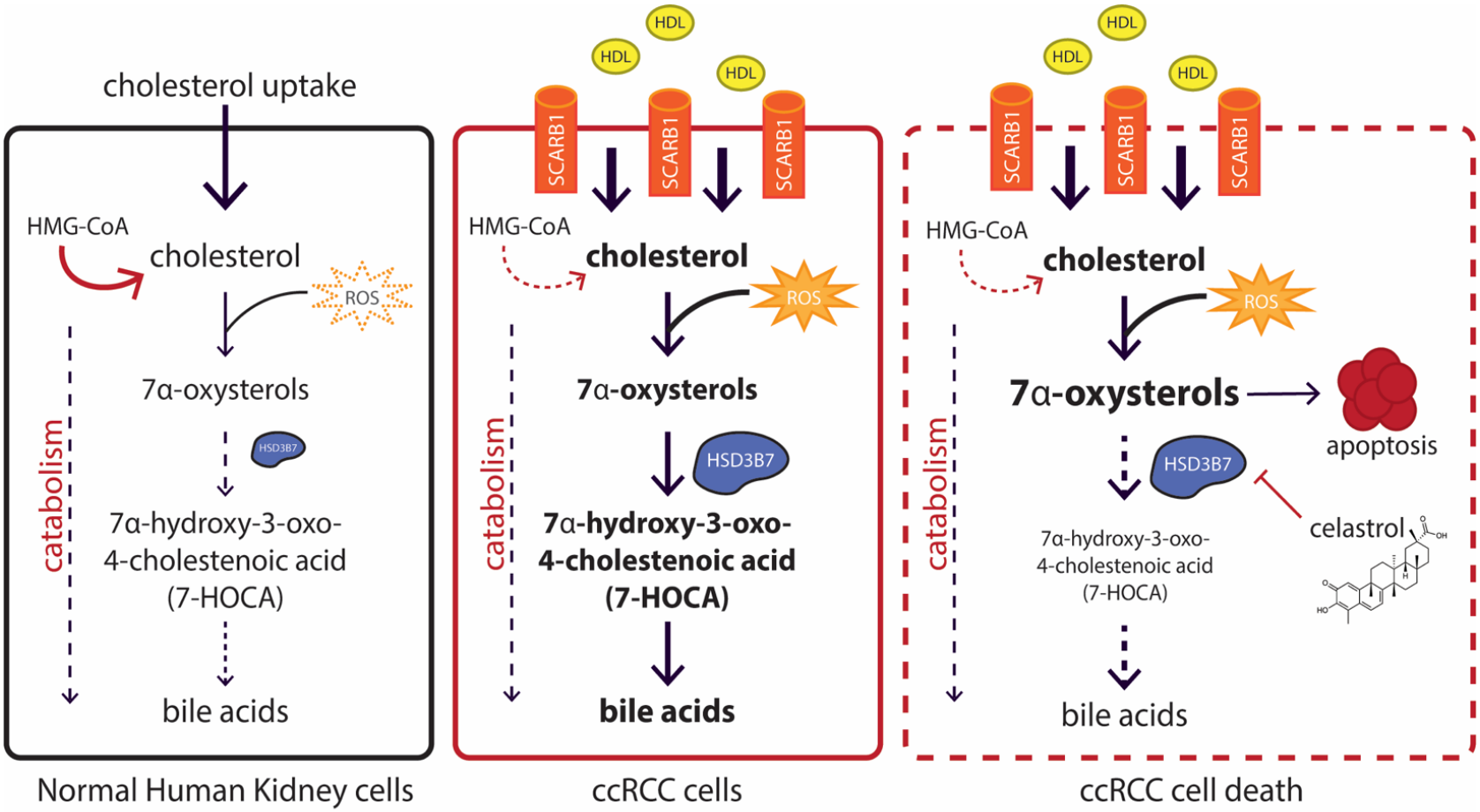
Take Home Message



Thanks for your attention

M. Celeste Simon
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Caroline Bull

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Foundation