Metabolic Alterations in Fumarate Hydratase Deficient Cells
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Graphical Abstract
Abstract: Mutations of the tricarboxylic acid cycle (TCA cycle) enzyme fumarate hydratase (FH) cause the hereditary cancer syndrome Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC). FH-deficient renal cancers are highly aggressive and metastasise even when small, leading to an abysmal clinical outcome. How these cells survive without FH and how they become transformed is still under investigation. Today, I will show our data on the metabolic reprogramming triggered by the loss of FH, which induces, amongst various changes, the fumarate-mediated succination of the iron-sulfur-cluster proteins ISCU1, NFU1, and Bola1/3. Of note, this post translational modification leads to defects in iron-sulfur cluster biogenesis and complex I deficiency. These results could help to explain the profound alteration of mitochondrial metabolism in cells that lack FH.

Keywords: cancer metabolism, fumarate hydratase, mitochondria
TCA cycle and cancer

Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer

The Multiple Leiomyoma Consortium
Published online: 25 February 2002; DOI: 10.1038/sj.bjc.6600660

Mutations in SDHD, a Mitochondrial Complex II Gene, in Hereditary Paraganglioma
Bora E. Baysal, et al.
Science 287, 848 (2000); DOI: 10.1126/science.287.5454.848
FH and HLRCC

Hereditary Leiomyomatosis and renal cell cancer
Skin fibroids
Uterine fibroids
Papillary type 2 renal cancer
Unanswered questions:

• How do these cells survive without FH?

• Why loss of FH leads to cancer?
Metabolic adaptations in cancer cell

NADH shuttling

Lipid metabolism

Urea cycle
PNC cycle

Haem biosynthesis

NADPH

Protein metabolism
Metabolic adaptations in cancer cell

Metabolic adaptations

Synthetic Lethality

NADH shuttling
Lipid metabolism
Urea cycle
PNC cycle
NADPH
Haem biosynthesis
Protein metabolism
The model: Fumarate Hydratase deficient (Fh1-/-) cells
Metabolic profile of Fh1-deficient cells

- Fh1+/+ vs Fh1−−

- Fumarate to malate conversion

- OCR and ECAR measurements for Fh1+/+ and Fh1−/− CL1 and CL19
Metabolic diversions in \( Fh1^{-/-} \) cells
Heam biosynthesis pathways in Fh1-deficient cells
Fumarate leads to succination of glutathione
Fumarate leads to reversal of Argininosuccinate Lyase
Mitochondrial dysfunction in Fh1-deficient cells
The models: $Fh1^{-/-}$ rescue cells

epithelial kidney cells $\xrightarrow{Fh1^{fl/fl}} Fh1^{-/-} \xrightarrow{+CRE} Fh1^{-/-}+Fh1$ low $\xrightarrow{+Fh1} Fh1^{-/-}+Fh1$ high
Fh1-rescue restores fumarate levels and respiration

Fh1
Assessing respiratory chain activity
Respiratory chain activity in Fh1-deficient cells

Complex I
- 5 mM Glutamate
- 5 mM Malate
- 2 μM Rotenone
- Fh1^{+/+}
- Fh1^{−/−}CL1
- Fh1^{−/−}CL19
- Fh1^{−/−}pFh1 high

Complex II
- 10 mM Succinate
- 2 μM Antimycin A

Complex III
- 0.5 mM Duroquinol
- 2 μM Antimycin

Complex IV
- 100 μM TMPD +
- 10 mM Ascorbate
- 20 mM Azide
Complex I activity in *Fh1*⁻/⁻ cells
Fumarate does not affect Complex I
Complex I levels are not decreased in *Fh1*<sup>−/−</sup> cells

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<th>Fh&lt;sup&gt;−/−&lt;/sup&gt; CL&lt;sub&gt;19&lt;/sub&gt;</th>
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*Note:*

- *Tmem1*<sub>126b</sub>
- *Ndufs12*
- *Ndufs7*
- *Ndufs16*
- *Ndufs4*
- *Ndufs11*
- *Ecox*
- *Ndufs18*
- *Sac19*
- *Ndufs9*
- *Ndufs41*
- *Ndufs25*
- *Ndufs6*
- *Ndufs11*
- *Ndufs10*
- *Ndufs2*
- *Ndufs5*
- *Tmem40c1*
- *Ndufs8*
- *Ndufs13*
- *Ndufs5*
- *Ndufs12*
- *Ndufs1*
- *Mitochondrial Ndufs*
- *Ndufs6*
- *Ndufs3*
- *Ndufs8*
- *Mitochondrial Ndufs*
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- *Ndufs4*
- *Ndufs5*
- *Ndufs2*
- *Ndufs11*
- *Ndufs9*
- *Ndufs10*
- *Ndufs7*
- *Ndufs3*
Protein succination and fumarate accumulation

High concentration of fumarate

protein

No enzyme

2-succin-cysteine adduct
Fe-S cluster biogenesis proteins are succinated in $Fh1^{-/-}$ cells
Fe-S cluster proteins and respiratory chain

Fe-S cluster biogenesis:
- NFS1
- ISCU
- Fe²⁺
- Cys

Fe-S cluster transfer:
- Nfu1
- Fe-S aconitase
- Fe-S
- CI
- 8Fe-S
Fe-S are decreased in Fh1-deficient cells

Hoff et al 2009

Venus GRX2 fluorescence (488/530nm)

mean fluorescence (Venus-positive cells)

+Venus GRX2

Fh1

Fh1+COL1

0 1000 2000 3000 4000 5000

10^1 10^2 10^3 10^4 10^5
Metabolic Alterations in Fumarate Hydratase Deficient Cells

- Glucose
- Lactate
- Pyruvate
- Alanine
- Cystine
- Glycine
- Serine
- 2SC
- Fumarate
- ASA
- Arginine
- Citrate
- Succinate
- Succinyl-CoA
- aKG
- OAA
- AcCoA
- GA3P
- R5P
- PRPP
- ISCU1
- NFU1
- Fe-S cluster biogenesis
- Complex I
- Aconitase
- Glutamine
- Glutamate
- Bilirubin
- Haem
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