Regulation of iron metabolism by sideroflexin SFXN1

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Introduction

SIDEROFLEXINS

- SFXNs are conserved in eukaryote
- Mammalian SFXNs → 5 members (SFXN1-5)
- Mitochondrial carriers (SLC56)
- SFXN1 main function = serine transporter
- SFXN1 potentially involved in iron homeostasis → controversial

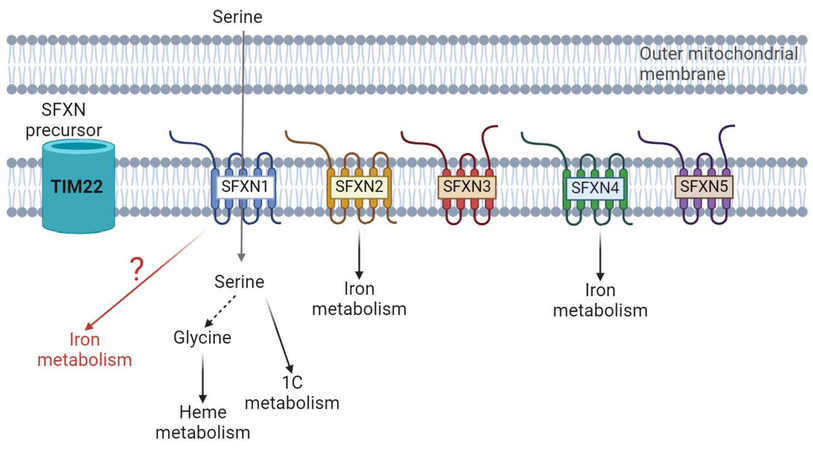


Fig 1. Functions of the mammalian sideroflexins (SFXN). Created with BioRender

FERROPTOSIS & signaling pathway

- Cell death driven by iron-dependent lipid peroxidation
- Ferroptosis is activated physiologically and pathologically
- Deregulation of iron homeostasis, ROS & glutathione metabolism trigger ferroptosis

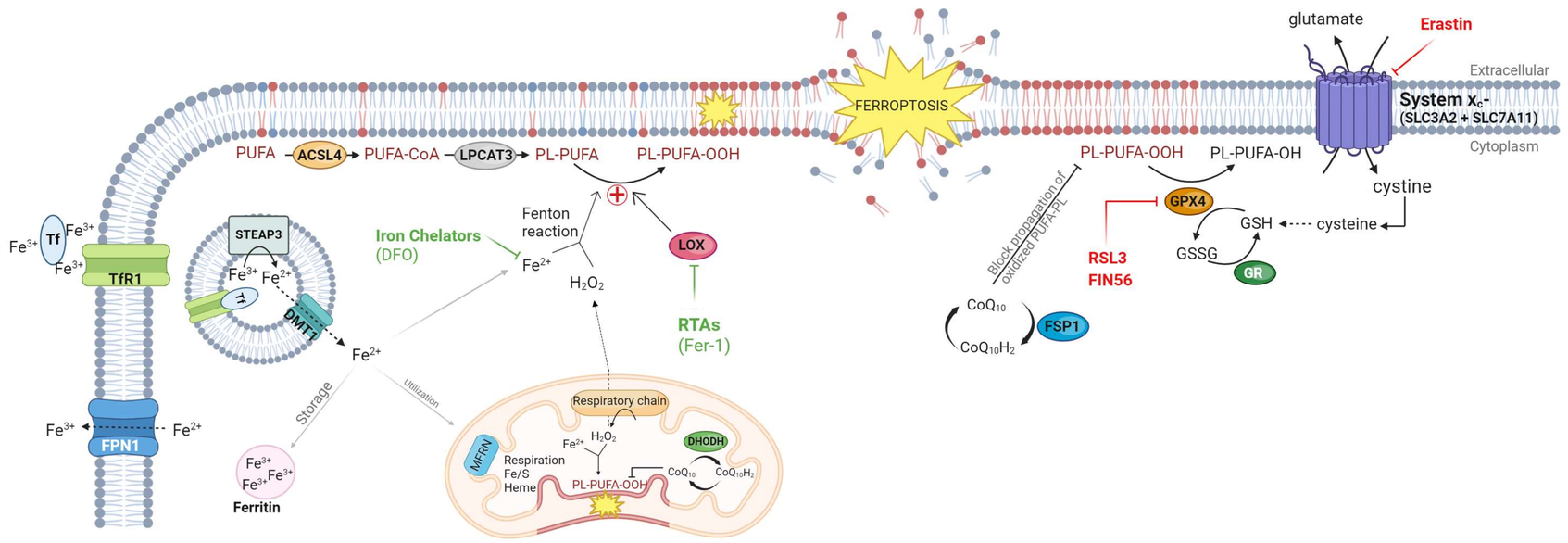
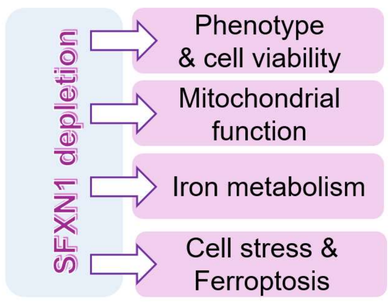


Fig 2. Mechanisms of ferroptosis. Ferroptosis inducers (red) and ferroptosis suppressors (green) are shown. Created with BioRender

Consequences of SFXN1 depletion on iron metabolism and ferroptosis ?

METHODS

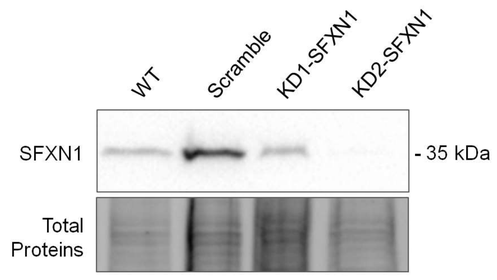
Scientific questions



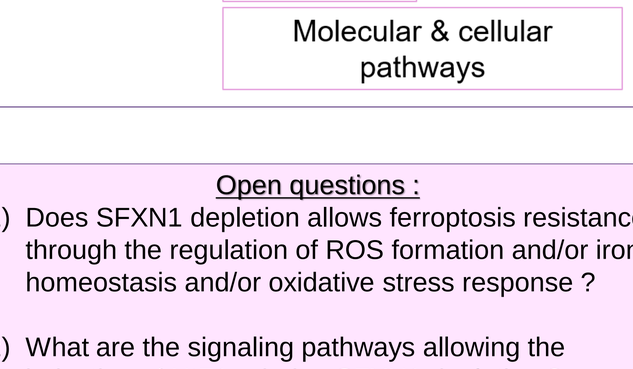
Cellular model

- HT1080 cancer cells (fibrosarcoma) sensitive to ferroptosis inducers
- Stable knockdown of SFXN1 (KD1 & KD2 – SFXN1)
  → 2 different shRNA targeting SFXN1 transcripts
- shRNA-Scramble as control

Verification by western-blot



CONCLUSION



Open questions :

1) Does SFXN1 depletion allows ferroptosis resistance through the regulation of ROS formation and/or iron homeostasis and/or oxidative stress response ?

2) What are the signaling pathways allowing the induction of HIF1α during SFXN1 depletion ?

References:
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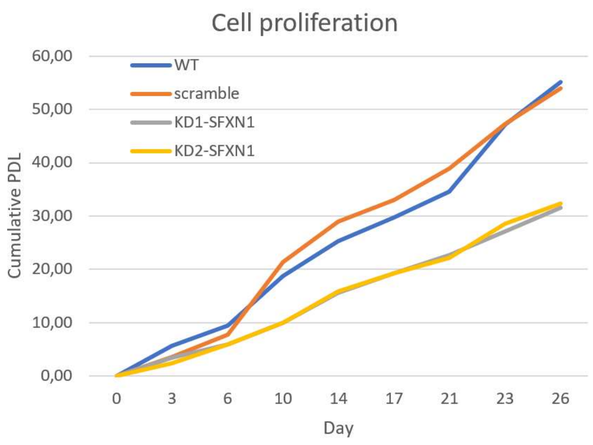
RESULTS

Cellular phenotype

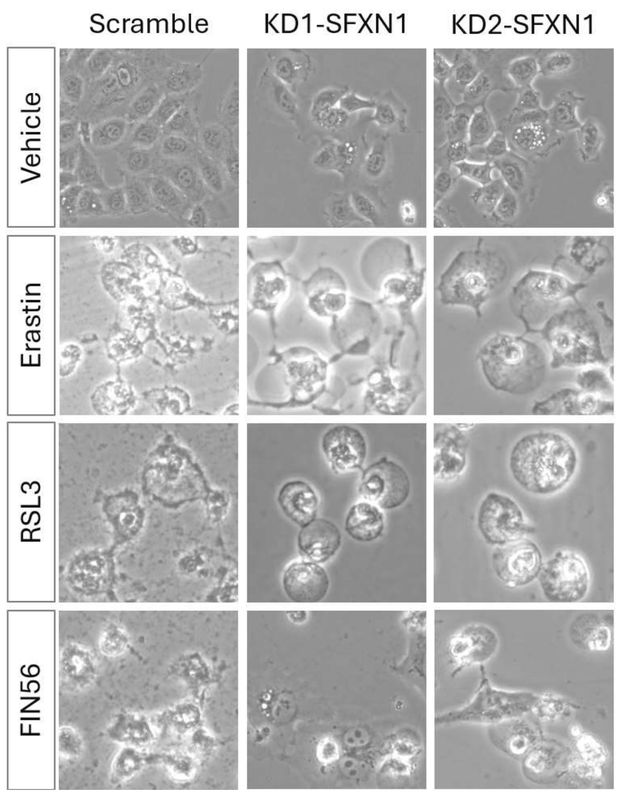


- KD cells proliferated slowly in full medium compared to control cells

→ Test the viability and cytotoxicity (stress oxidant condition)

Ferroptosis sensibility

Cellular morphology



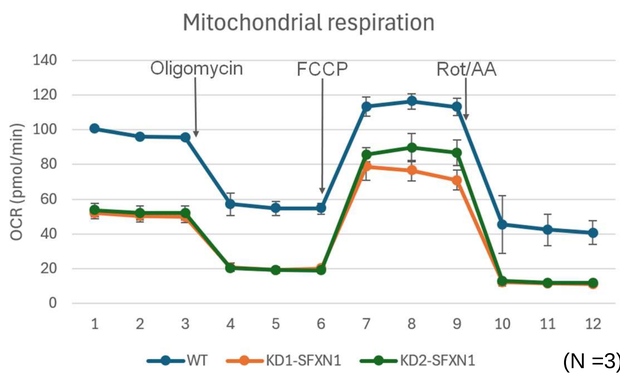
24H treatment with 10µM of erastin, 1µM of RSL3 or 5µM of FIN56

- Delay of ferroptosis, KD cells appear to be resistant (or less sensitive to ferroptosis inducers)

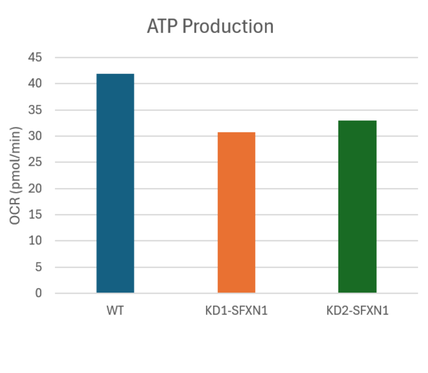
→ What are the mechanism allowing this resistance ?

Mitochondrial function & morphology

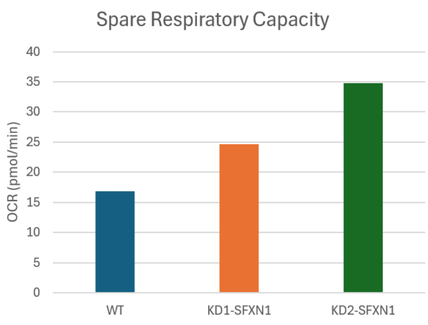
Mitochondrial respiration by Seahorse



(N =3)



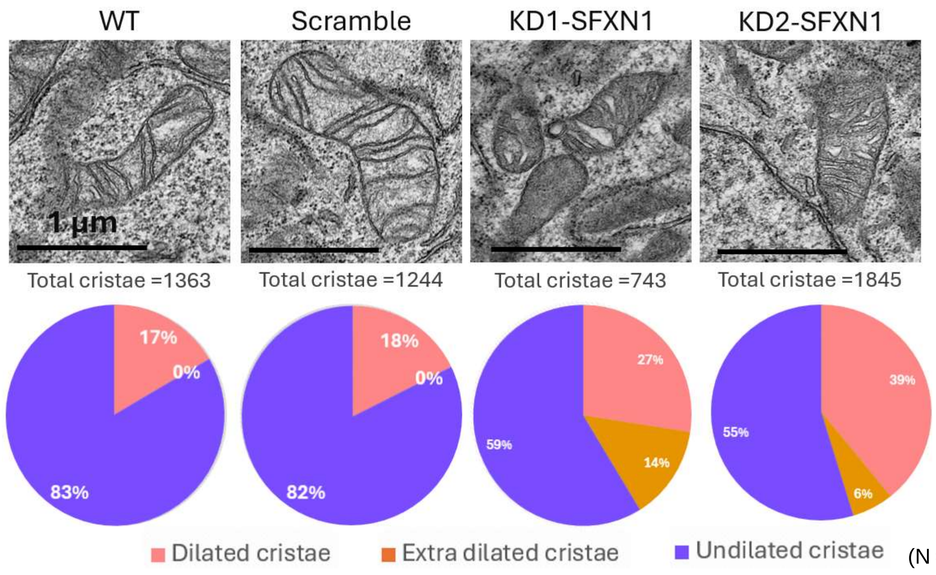
- Oligomycin inhibits ATP synthase
- FCCP uncoupling agent = disrupts mitochondrial membrane potential
- Rotenone / Antimycin (Rot/AA) inhibits complex I and III



- SFXN1 depletion seems to decrease the mitochondrial respiration
  - Decrease of ATP production
  - Increase of spare capacity

→ Mitochondrial function alteration results from a disorganization of respiratory complexes ? Problem with complex activity ? Alteration of mitochondrial morphology ?

Mitochondrial morphology by TEM



(N =2)

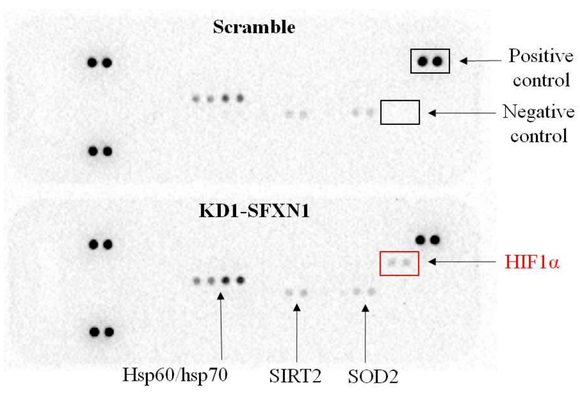
- Altered cristae morphology in KD cells

→ Test mitochondrial biogenesis and mitochondrial network

→ Investigate the composition of the complexes maintaining cristae junctions

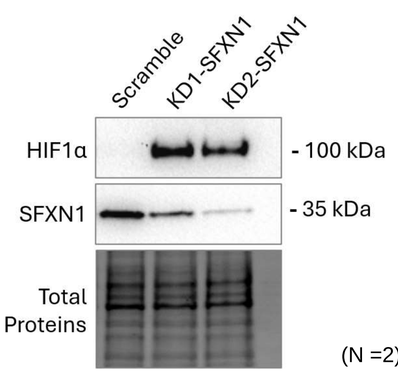
HIF1α is induced upon SFXN1 depletion

Proteome Profiler Array (cell stress kit)



- Same results with KD2-SFXN1

HIF1α by western-blot



(N =2)

- HIF1α seems to be upregulated in KD cells

→ Iron can regulate HIF1α, so does SFXN1 can regulate HIF1α through iron ?