

Exploring Citrin as a Therapeutic Target for Cancer Treatment Through a Clinical, Pharmacologic, and Metabolic Lens

INTRODUCTION

Metabolic reprogramming in cancer

- **Metabolic reprogramming** is a **double-edged sword**: cancer cells alter metabolic pathways to **support proliferative needs** → **therapeutic vulnerability**
- Aspartate is an essential precursor in **pyrimidine synthesis** pathway → essential for cancer proliferation

Citrin metabolic importance

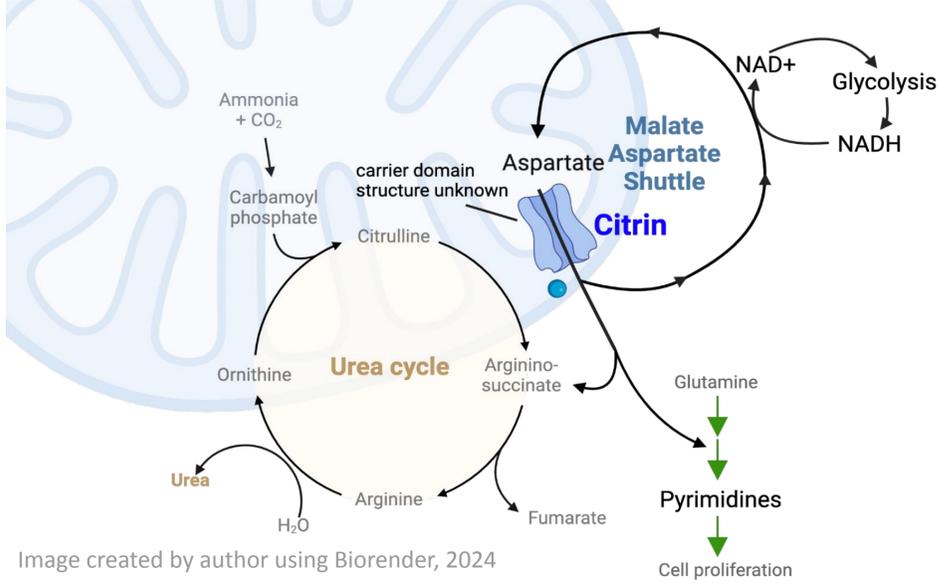


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- **Citrin (SLC25A13)** → mitochondrial carrier responsible for aspartate + glutamate transport
 - Important in **urea cycle, malate-aspartate shuttle, & aspartate transport**— pathways rewired in cancer cells
 - Germline mutation leads to **Citrin Deficiency**
 - **Full protein structure unknown**

Citrin may be important in cancer

- Rabinovich et. al 2015 identify Citrin as an essential source of cytosolic aspartate in ASS1-deficient osteosarcoma cells
- Rabinovich et. al 2019 finds **Citrin overexpression in melanoma cells** to correlate with **increased migration *in vitro***

METHODOLOGY

Research Question How does Citrin function impacts cancer patient prognosis, how Citrin can be pharmacologically targeted, and how does Citrin targeting metabolically alters cancer cells?

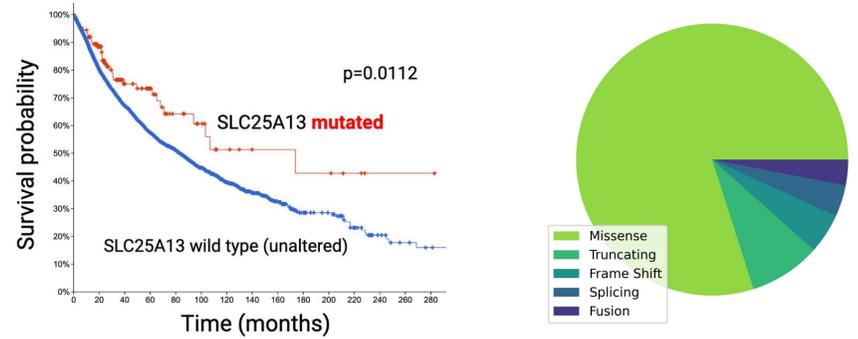
- 1 Analyze public de-identified cancer patient database for importance of Citrin mutations
- 2 Computational model Citrin structure and transport mechanism using AF2 and docking
- 3 Analyze structural and functional impact of Citrin missense mutations found in cancer patients
- 4 Dock FDA approved drugs (>1600) to identify drugs capable of being repurposed for Citrin inhibition
- 5 Analyze metabolic impact of CRISPR Cas-9 Citrin knockdown in colon cancer cells

CONCLUSIONS

- Creation of first complete **Citrin structural model**
 - Insight into protein movement mechanisms important for both cancer and Citrin deficiency patients
- Citrin missense mutations likely lead to loss of protein function → **loss of Citrin function is correlated with better patient survival**
- Identified **FDA-approved drugs** repurposable as **Citrin inhibitors**
- Citrin knockdown in HCT116 cells significantly decreases asparagine, proline, and citrate levels
 - Citrin knockdown impact on pathways downstream of cytosolic aspartate suggests **Citrin inhibition may be a promising target for decreasing cytosolic aspartate levels**
- Future directions: Assess Citrin inhibition *in vitro* and *in vivo*
 - Use Citrin deficiency treatments (low carbs, MCT) to reduce normal cell toxicity

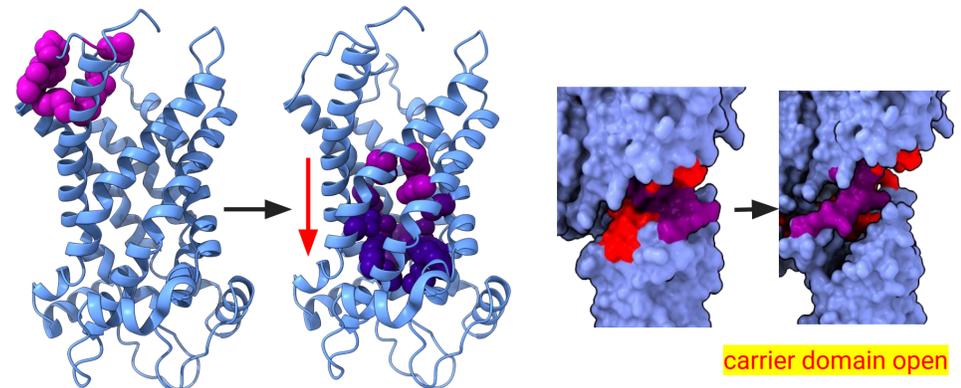
RESULTS/ DISCUSSION

Citrin mutations are associated with favorable cancer patient outcome



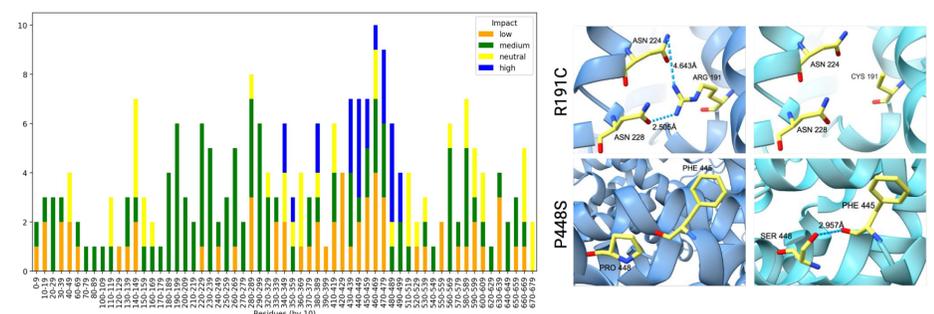
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AF2 model reveals possible substrate transport and regulation mechanism



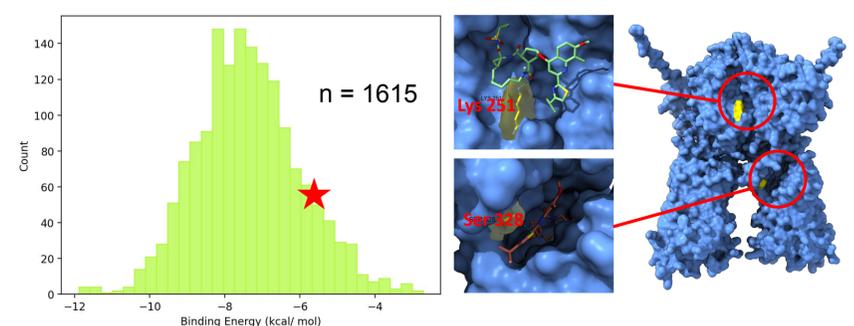
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Missense mutations likely lead to loss of Citrin protein function



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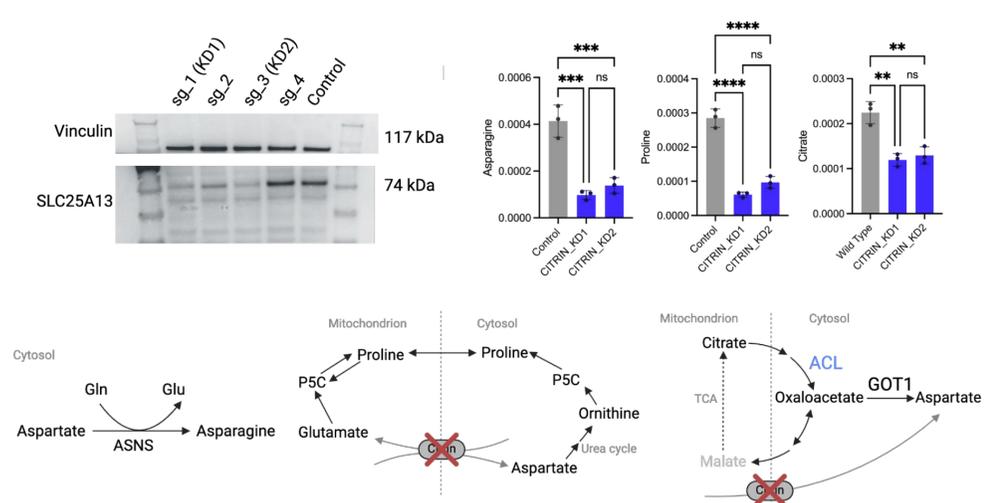
Potential Citrin-inhibiting FDA-approved drugs identified



Ligand	Source	Binding energy (Kcal/mol)	Inhibition Constant μM $K_i = \exp(\Delta G/RT)$
Simeprevir (Olysio)	ZINC000164760 874	-11.9	.00192
L-Aspartate	Pubchem CID: 5460541	-5.4	109

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Citrin knockdown in colon cancer cells suggests decreased cytosolic aspartate availability



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